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	PAT. NO.	Title
1	6,596,866	Process for the preparation of nefazodone hydrochloride
2	6,595,576	Housing for member disposed at vehicle exterior
3	6,595,412	Method for calculating indicia for mailpieces
4	6,595,090	Tool for manually turning an engine
5	6,593,960	Multi-functional on-vehicle camera system and image display method for the same
6	6,593,801	Power down mode signaled by differential transmitter's high-Z state detected by receiver sensing same voltage on differential lines
7	6,593,166	Method for construction of nanotube matrix material
8	6,592,692	Energetic plasticizer comprising bis(2,2-dinitropropyl) formal and bis(2,2-dinitropropyl) diformal, and preparation method thereof
9	6,592,249	Device for producing and/or processing mixtures consisting of multiple constituents
10	6,591,783	Marine life packaging and acclimatization system
11	6,590,979	Method and apparatus for compression compatible video scrambling
12	6,590,946	Method and apparatus for time-warping a digitized waveform to have an approximately fixed period
13	6,590,095	Method of preparing a cellulose carbamate solution
14	6,589,955	Pediatric formulation of gatifloxacin
15	6,589,781	Farm composting system
16	6,589,562	Multicomponent biodegradable bioadhesive controlled release system for oral care products
17	6,589,176	Ultrasonic image stabilization system and method
18	6,585,918	Process for modifying a uniformity of a tire
19	6,584,711	Method and apparatus for spreading a rectangular sheet of fabric

20 6,583,010 Trench transistor with self-aligned source
21 6,580,069 Atom probe
22 6,579,003 Decorative hourglass with light reflecting base
23 6,578,454 Machining process for hydrodynamic bearing
24 6,577,798 Method for producing non-linear optical organic crystal film
25 6,577,488 Inductive load driver utilizing energy recovery
26 6,577,140 Method and device for measuring the acidity or basicity of insulating fluids, particularly mineral and synthetic oils
27 6,577,127 Magnetic resonance imaging method for imaging time-dependent contrast
28 6,576,276 CO2-hydrate product and method of manufacture thereof
29 6,576,207 Oxygen storing material with high thermal stability and a process for preparation and use thereof
30 6,575,284 Clutch of a beach buggy
31 6,573,768 Power-on circuit of a peripheral component
32 6,573,213 Metal catalysts
33 6,571,993 Apparatus for holding and metered dispensing of an active composition into a washing machine, a laundry dryer or a dishwashing machine
34 6,571,665 Cutting instruments
35 6,570,413 Driver circuit for switching device
36 6,570,389 Prevention of arcing in power supplies
37 6,570,109 Quick shut-off extended range hygroscopic rain sensor for irrigation systems
38 6,570,014 Process for preparing triazolopyrimidine derivatives
39 6,569,923 Polymer-cement composites and methods of making same
40 6,569,468 Cinnamomi and poria composition, method to prepare same and uses thereof
41 6,568,514 Damping mechanism having a pressure actuated orifice seal and a compact internally disposed pressure relief assembly
42 6,567,752 General method for tracking the evolution of hidden damage or other unwanted changes in machinery components and predicting remaining useful life
43 6,567,262 Liquid cooled TEC based system and method for cooling heat sensitive elements
44 6,566,417 Contact lens having improved dimensional stability
45 6,565,982 Transparent multilayer device
46 6,565,901 Quick-setting gel mix
47 6,565,884 Bone graft material incorporating demineralized bone matrix and lipids
48 6,565,552 Partial aortic occlusion devices and methods for cerebral perfusion augmentation
49 6,564,689 Blank for gun barrel, method for producing said gun barrel and gun barrel
50 6,564,544 Engine exhaust purification arrangement

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Refine Search isd/19900101->20030728 and aclm/slowly and ccl/42

	PAT. NO.	Title
1	6,589,562	Multicomponent biodegradable bioadhesive controlled release system for oral care products
2	6,569,468	Cinnamomi and poria composition, method to prepare same and uses thereof
3	6,565,884	Bone graft material incorporating demineralized bone matrix and lipids
4	6,562,826	Sustained release ranolazine formulations
5	6,548,084	Controlled release compositions
6	6,544,515	Acceleration of the rate of digestion of a protein
7	6,540,986	Sunscreen compositions
8	6,534,487	Methods for suppressing appetite and enhancing exercise and recovery
9	6,521,247	Dual iron containing nutritional supplement
10	6,506,397	Pest controlling
11	6,497,900	Effervescent base
12	6,488,948	Anti-bacterial composition and use thereof for skin care and fabric treatment
13	6,485,950	Isozyme of autoclavable superoxide dismutase (SOD), a process for the identification and extraction of the SOD in cosmetic, food and pharmaceutical compositions
14	6,468,553	Formula and preparation method of an improved ointment for treating burns and scalds
15	6,468,551	Cosmetic or dermatological preparations based on emulsifiers which are free from ethylene oxide and propylene oxide, for the preparation of microemulsion gels
16	6,464,961	Polymeric delivery and release systems for oral care actives
17	6,423,348	Anticoagulant compositions
18	6,416,795	Herbal extract composition for stress prevention and treatment
19	6,399,092	Anhydrous, hydrophilic absorbent wound dressing (tube) with antimicrobials or other

pharmaceutically active agents

20 6,383,515 Solvent system for enhancing solubility

21 6,372,238 Method of using implants to fertilize, control growth and fungal and insect attack on banana or plantain

22 6,362,225 Target therapies for treating common viral infections

23 6,350,458 Mixed micellar drug deliver system and method of preparation

24 6,350,438 Oral care compositions comprising chlorite and methods

25 6,348,335 Low-molecular active weight ingredient extract from yeasts and method for producing it

26 6,344,188 Wrinkle reducing cream

27 6,342,250 Drug delivery devices comprising biodegradable protein for the controlled release of pharmacologically active agents and method of making the drug delivery devices

28 6,326,022 Slow-release disposable elastomeric buccal devices

29 6,322,822 Biocidal applications of concentrated aqueous bromine chloride solutions

30 6,316,008 Combination of zinc ions and vitamin C and method of making

31 6,315,987 Polymeric delivery and release systems for oral care actives

32 6,306,243 pH-modified biocompatible monomer and polymer compositions

33 6,303,102 Cutaneously applied biodegradable tell-tale having controllable clearing time

34 6,296,838 Anti-fungal herbal formulation for treatment of human nails fungus and process thereof

35 6,290,989 Expandable gastro-retentive therapeutic system with controlled active substance release in the gastro-intestinal tract

36 6,231,882 Mixed micellar delivery system and method of preparation

37 6,221,395 Controlled release pharmaceutical tablets containing an active principle of low water solubility

38 6,221,378 Mixed micellar delivery system and method of preparation

39 6,207,153 Antigen binding fragments that specifically detect cancer cells, nucleotides encoding the fragments, and use thereof for the prophylaxis and detection of cancers

40 6,193,994 Locally administrable, biodegradable and sustained-release pharmaceutical composition for periodontitis and process for preparation thereof

41 6,175,035 Method of producing betulinic acid

42 6,159,480 Cosmetic makeup composition

43 6,153,181 Granular pest bait

44 6,143,352 pH-modified biocompatible monomer and polymer compositions

45 6,129,931 Controlled-release, drug-delivery tableted composition including a complex between poly(maleic diacid-alkyl vinyl ether) and polyvinylpyrrolidone

46 6,117,852 Boron-containing lipiodol for boron neutron capture therapy of hepatoma

47 6,068,854 Sol-controlled thermocolloid matrix based on gelatin for oral sustained-release form

48 6,034,175 Salts of peptides with carboxy-terminated polyesters

49 6,017,545 Mixed micellar delivery system and method of preparation

50 5,994,406 Geminal cationic surfactants of the type N.alpha. N.omega. bis (N.alpha.-acyl-arginin) .alpha.,.omega. diamino alkyl dichlorohydrates as antimicrobial agents

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US006423348B1

(12) **United States Patent**
Mickus

(10) **Patent No.: US 6,423,348 B1**
(45) **Date of Patent: Jul. 23, 2002**

(54) **ANTICOAGULANT COMPOSITIONS**

(76) Inventor: **James C. Mickus**, 5269 Heritage Hills Dr., Bloomington, MN (US) 55437

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/212,221**

(22) Filed: **Dec. 15, 1998**

(51) **Int. Cl.**[7] **A01N 59/26**; A01N 37/36; A01N 37/44

(52) **U.S. Cl.** **424/606**; 424/601; 424/602; 424/603; 424/605; 424/688; 424/690; 424/692; 424/693; 424/722; 514/557; 514/566; 514/574; 514/822; 436/18

(58) **Field of Search** 424/602, 603, 424/688, 690, 692, 693, 601, 605, 606, 722; 514/566, 557, 574, 822; 436/18

(56) **References Cited**

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3,947,919 A	*	4/1976	Ekdahl	452/68
4,090,977 A		5/1978	Dubin	
4,270,241 A	*	6/1981	Braga	452/69
4,529,614 A		7/1985	Burns	
4,788,139 A		11/1988	Ryan	
5,556,643 A		9/1996	Bohannon et al.	

FOREIGN PATENT DOCUMENTS

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Hompson, Daniel U., & Weaver, Eric M.—USDA Inspected, Edible, Blood PRoteins in Calf Milk Replacers (continued) American Protein Corp. Discoveries, Winter 1998 published on the world wide web —Dec. 14, 1998.

* cited by examiner

Primary Examiner—John Pak
(74) *Attorney, Agent, or Firm*—Larry M. Jennings, P.A.

(57) **ABSTRACT**

What is shown is an animal blood anticoagulant compound useful in the meat packing industry generally, and in slaughterhouse operations, particularly. The anticoagulant is effective when diluted with water at higher dilution ratios than earlier anticoagulants. In some field trials, this anticoagulant was at least as effective as previously known commercial anticoagulants when diluted by an additional 30%. The present anticoagulant preparation concentrate is an aqueous mixture of soft water (55.0%–65.0/%, w/w); tetrasodium ethylene diamine tetraacetate (Na_4EDTA) (0.5%–3.0%, w/w); sodium hexametaphosphate (17.0%–24.0%, w/w); citric acid (5.0%–9.0%, w/w); and sodium hydroxide (4.0%–7.0%, w/w) to obtain a balanced pH that provides optimal chelating and anticoagulant activity. Optimal anticoagulant performance has been found to occur in the range of between pH 6.6 and pH 7.2. The degree to which the product may be diluted varies with the characteristics of the facility, the diluent water, the species of animal blood being treated, and the breed of the species, among other things.

19 Claims, No Drawings

ANTICOAGULANT COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates to the field of anticoagulant compositions, generally, and to anticoagulant compounds adapted for use in slaughterhouses, more particularly. A method of making improved anticoagulant compositions is disclosed. In addition, a method of using anticoagulant compositions is described. Most specifically, the present disclosure teaches an improved method by which a selected mixture of chelating agents, or sequestering agents, may be used in a slaughterhouse as an animal blood anticoagulant.

BACKGROUND OF THE INVENTION

Anticoagulant compositions are used in slaughterhouse operations to permit the recovery of blood from the work area. In particular, the area where animals are killed and bled must be cleaned frequently to prevent the rapid accumulation of coagulated blood which could be removed only with great difficulty.

Blood from slaughter animals was traditionally viewed as a low value component that is sometimes discarded or used for fertilizer. There is, however, an improving market for various whole blood constituents. Dried animal plasma proteins may be purified and used as a protein additive in many products, including confectionary and other food products for human consumption. In addition, dried animal red blood cells (RBC) are routinely added to dark breads in some European countries.

Edible blood products can be produced for commercial use can be prepared from materials produced by meat-packing slaughter operations and other facilities such as poultry kill plants that are suitably inspected and monitored for food safety. Spray-dried whole blood, powdered red blood cells, spray-dried plasma, and serum protein can be incorporated into calf milk replacer products. Spray-dried plasma is a tan-colored, free-flowing powder that is approximately 78% (seventy-eight percent) protein.

In the absence of preventive measures, blood from slaughtered animals will coagulate quickly after being exposed to air. The accumulation of coagulated blood may be avoided by washing the area with large quantities of water, but doing so greatly diminishes the value of the product by greatly increasing the volume of liquid that must be processed. It is readily appreciated that separation of the desirable constituents of slaughterhouse blood from the undesirable ones (the most voluminous of which is water) may be carried out more efficiently if the concentration of desired constituents is high.

Techniques analogous to those of the milk processing industry can be used in collecting the blood of slaughter animals, storing it safely until it can be processed, and then processing it into desired fractions or products. For example, slaughter animals can be positioned so that the blood flows from the animal directly into stainless steel troughs, basins, channels, and conduits. An anticoagulant composition can be applied immediately, usually by spraying continuously onto the surface of a receiving trough situated below the animals. The anticoagulant keeps the blood in liquid form until the desired separation, purification, concentration, or other processing steps are performed. The treated blood can flow by gravity to a suitable collection or storage receptacle from which it may be pumped or hauled to a different location for processing.

Effective anticoagulant application broadens the selection of processing methods that may be selected to prepare

desired products. Among other commonly used processing techniques, the treated blood may be separated using filtration or centrifugation; dried by a variety of processes including spray drying, drum drying, and freeze drying; or concentrated and purified using reverse osmosis. Products intended for human consumption, pharmaceutical, or laboratory applications are frequently processed with reverse osmosis. Dried RBC, liquid or dried plasma protein are prepared in substantial volume, particularly for use as protein supplements in animal feeds, often mixed with proteins or selected amino acids from other sources. Blood protein may also be mixed with other proteins and formed into feeds or feed supplements.

Offsetting the benefit of the higher concentration of blood solids in the collected material is the expense of purchasing the anticoagulant composition. Anticoagulant cost is an important consideration in determining which, if any, product to use. A single, modestly sized, meat packing facility might use 3,500 or 4,000 gallons of anticoagulant a week. The anticoagulant consumption rate is substantial and represents a significant portion of the cost of the products that are ultimately marketed.

Previously known anticoagulant preparations that have relatively low potency or anticoagulation activity can have higher handling costs as a direct result. Storing, conditioning, shipping, handling, pumping, and administering anticoagulant preparations comprise significant costs. When lower activity anticoagulant preparations result in the necessity of using greater volumes of those materials, handling costs can be expected to increase. The fact that blood is a low value product makes it necessary to consider and control the costs of converting that product into marketable products. Anticoagulant application, handling, properties, and efficacy directly affect many of the steps required in the processing of animal blood.

Another consideration is the effect of the anticoagulant upon other components of the blood handling system. Blood products must be filtered regardless of which anticoagulant is used. Accumulations of coagulated blood particles tend to build up in system filtration media at rates that depend, at least in part, on the anticoagulant used. Once the filtration media has become fouled with coagulated blood, it must be cleaned to resume operation. Similarly, the performance of centrifuges that are used to separate cellular particles from liquids containing dissolved solids is adversely affected by the accumulation of residue that adheres to the separating surfaces of the centrifuges. Much of the residue that accumulates on filters, in centrifuges, and on other blood handling system surfaces is coagulated blood that must be periodically removed in order to keep the system operating. Cleaning the blood handling system components is laborious and time-consuming. System component materials, anticoagulant preparations, and other additives, that have the likelihood of reducing the amount of time required for cleaning centrifuges, pre-filters, bag filters, and other system elements are sought to improve efficiency and reduce the cost of obtaining the desired blood fractions and other products.

Several anticoagulant compositions have been developed for various applications that include preservation of diagnostic samples, among others. Heparin, Alsevers Solution, sodium citrate, and EDTA are commonly available anticoagulant laboratory reagents.

Ethylene diamine tetraacetic acid (EDTA) has been successfully used as a chelating agent for a variety of purposes, and as an anticoagulation agent in laboratory settings, pri-

marily in connection with diagnostic blood testing ordered in conjunction with medical and surgical procedures. For example, U.S. Pat. No. 4,090,977 issued to Dubin May 23, 1978 for his Osmotically Balanced Anticoagulant. Dubin made a preservative for whole blood comprised of an admixture of the free acid form of EDTA and an alkali metal salt of EDTA to obtain desired hematocrit values.

U.S. Pat. No. 4,529,614 to Burns issued Jul. 16, 1985 for a One Step Anticoagulant Coating. The coating described by Burns contains silicone and EDTA; it is designed to be applied to the inner surfaces of plastic and glass blood test vessels to make the treated surfaces hydrophobic and also to prevent coagulation.

Ryan received U.S. Pat. No. 4,788,139 Nov. 29, 1988 for his Platelet Aggregation Reagent, Reagent Container and Method of Determining Platelet Aggregation in EDTA-Anticoagulated Blood. His reagent overcomes the anticoagulation properties of EDTA in a blood sample and allows platelet aggregation of the sample to be evaluated.

U.S. Pat. No. 5,556,643, entitled Anticoagulant Compositions, issued Sep. 17, 1996 to Bohanon, et al. and is directed to products that can be used to treat animal blood in slaughterhouses. The Bohanon et al. anticoagulant contains sodium hydroxide (NaOH), citric acid and sodium hexametaphosphate.

A commonly encountered problem with the presently available commercial anticoagulants is that the compositions tend to build up on centrifuge walls and foul filters and other processing equipment. An anticoagulant composition having a reduced tendency to build-up on centrifuge walls and other components would be a distinct advantage.

Sodium citrate, when used alone as an anticoagulant, must be applied at relatively high concentrations, in the range of 6% to 8% by weight. A consequence of the addition of large amounts of sodium citrate is that the ash content of the resulting product may be substantially higher than if anticoagulants having greater activity are used. Increased ash restricts the uses for which the protein product is suited even though the additional material may be inconsequential in other instances.

SUMMARY OF THE INVENTION

What is needed is an anticoagulant for use in slaughterhouses that, compared to known anticoagulants, gives better cost performance. A further need is for animal blood anticoagulant that has less tendency to cause build up on the surfaces of centrifuges and other blood processing equipment, including, without limitation, filters conduits, and heat exchangers. Yet another need is for versatile, reliable, animal blood anticoagulant compounds that are effective in relatively low concentrations to minimize the amount of ash that is added to the finished products. A still further need in the art is for an anticoagulant composition that is effective at lower concentrations to minimize the dilution of whole blood caused by the addition of anticoagulant.

The present invention overcomes the limitations of the anticoagulant compositions known in the art. Embodiments according to the present disclosure meet the needs of those who use animal blood anticoagulants in the course of preparing fractionated blood products.

An embodiment of anticoagulant according to the present disclosure can be prepared using a mixture of chelating agents or sequestering agents dissolved in soft water and the pH adjusted with an alkali metal hydroxide to a range of between 6.0 and 8.0, and more preferably, to a pH range of between 6.6 and 7.2. Chelating agents found to perform satisfactorily in combination include tetrasodium EDTA, citric acid, and sodium hexametaphosphate. Although any alkali metal hydroxide can be used in the present anticoagulant, it has been found that sodium hydroxide and potassium hydroxide are more desirable than are hydroxides made from other elements of the group.

It is possible to use the anticoagulant mixture described above in a method of reducing the unwanted coagulation of animal blood in slaughterhouses comprised of the steps of suspending a freshly killed animal from a moving conveyor above a blood collection trough, the blood collection trough having an elongated axis directly below and parallel to the direction of conveyor movement, draining the blood from the animal into the trough while the conveyor moves, spraying anticoagulant onto the surface of the trough and blood, the anticoagulant consisting essentially of an effective amount of tetrasodium EDTA with, an aqueous solution containing citric acid in the range of between about 0.40% and about 20%, by weight, alkali metal hexametaphosphate in the range of between 1% and 6% by weight, and sodium hydroxide to adjust the pH to within the range of between pH 6.6 and pH 7.2. The method may use anticoagulant spray that is prepared from a concentrated solution and diluted with soft, de-mineralized, distilled, or de-ionized water by a dilution factor in the range of between 4:1 and 20:1. In some cases, it may be advantageous to dilute the mixture in a batch process prior to spraying. In other instances, it may be preferred to mix the concentrated anticoagulant with the diluent using venturi extraction or metering pumps with in-line mixing.

Accordingly, it is an object of the invention to provide an anticoagulant composition that is effective at lower concentrations than are commercially available anticoagulants currently used at slaughterhouses to keep animal blood liquid.

It is a second object of the invention to provide a method of making an anticoagulant composition that is effective at lower concentrations than are the anticoagulants presently available commercially.

It is a third object of the invention to disclose a method for using an anticoagulant composition in slaughterhouses to prevent blood of slaughtered animals from coagulating before it can be processed and separated into various useful products.

It is a fourth object of the invention to provide an anticoagulant preparation that, compared to previously known commercial anticoagulant products, has an equivalent anticoagulant activity at a dilution ration that is 15% to 20% higher than has been possible before the presently disclosed composition.

It is a fifth object of the invention to provide an aqueous anticoagulant preparation that may be used on avian, bovine, porcine and other species.

It is a sixth object of the invention to provide a non-frothing aqueous anticoagulant for applying to animal blood in slaughterhouses.

It is a seventh object of the invention to provide an anticoagulant that will result in the formation and deposition of less residue on centrifuge walls.

It is an eighth object of the invention to provide an anticoagulant that will increase the length of time that filters and other equipment can operate between required equipment cleaning procedures.

It is a ninth object of the invention to provide an anticoagulant that has improved storage characteristics, better

temperature stability, and the property of withstanding repeated freeze-thaw cycles without performance degradation and without impairment of either the physical or the visual properties of the product.

Another objective is to provide a composition with optimal chelating activity.

Another object of the invention is to provide an anticoagulant solution having a pH that is optimal for chelating activity and in which chelating agents included in the composition are optimally active.

It is also an object of the invention to provide a packaged animal blood anticoagulant composition sufficiently concentrated that it will perform satisfactorily when diluted by the user with water to a greater dilution factor than is possible to use with known anticoagulants.

It is another object of the invention to provide an anticoagulant that is miscible with water in all proportions.

A further object is to provide an anticoagulant of which a substantial portion is made using soft water.

It is yet another object of the invention to provide anticoagulant preparations that are more cost-effective than are anticoagulant products presently available in the marketplace.

It is still another object of the present invention to disclose a method of manufacturing an anticoagulant that has better cost performance than do anticoagulants manufactured using previously known methods.

Another object of the invention is to provide an anticoagulant that is suitable for use in products intended for human consumption.

It is another object of the invention to provide an anticoagulant that can be manufactured readily at low cost using conventionally available processing and blending equipment.

These and other objects are satisfied by an embodiment of the present disclosure as more fully set out in the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention as well as its objects and advantages can be illustrated by describing the new anticoagulant composition, the method of producing the new anticoagulant composition, and the method for using the new anticoagulant composition.

My new anticoagulant composition for use in animal blood is an aqueous mixture of gras (generally recognized as safe) materials, tetrasodium ethylene diamine tetraacetate (Na_4EDTA), hexametaphosphate, and citric acid, balanced to a pH that yields optimal activity. Although the composition may exhibit anticoagulant activity when the pH is in the range between 5.5 and 8.5, it is believed performance is enhanced when the pH of the solution is in the range between 6.0 and 8.0. More preferably, it appears that optimal anticoagulant performance occurs in the range of between pH 6.6 and pH 7.2.

The proportions of the ingredients were determined empirically with the aim of obtaining maximum chelating performance. The ingredients of this anticoagulant composition may be provided in the following proportions:

INGREDIENTS	% BY WEIGHT
Tetrasodium EDTA	0.5–3.0
Citric Acid	5.0–9.0
Sodium Hexametaphosphate	17.0–24.0
Sodium Hydroxide	4.0–7.0
Water	55.0–65.0

The weight percentages shown above are those considered optimum and are not to be construed as limitations. It is to be understood that some or all of the weight percentages shown could be varied by 25% or more and still obtain satisfactory anticoagulant characteristics. In addition, the weight percentages will necessarily change when one equivalent ingredient is substituted for another. For example, if potassium hexametaphosphate $(KPO_3)_6$ is substituted for the sodium hexametaphosphate $(NaPO_3)_6$ the formula weight would change from 611.17 to 708.44, an increase of 16%. It would be necessary for that reason to replace 24 pounds of the $(NaPO_3)_6$ with about 28 pounds of the $(KPO_3)_6$ to maintain the same activity level.

The present anticoagulant product is made by dissolving the citric acid completely in a portion of the water and then adding the Na_4EDTA with vigorous agitation. It is preferred to use water that is void of hardness to obtain optimum anticoagulant activity. Embodiments of the present disclosure may be prepared using soft water, de-ionized water, distilled water, or de-mineralized water, all of which are deemed equivalent for the objects hereof. It is anticipated that the cost of softened water will be less than the cost of water that is made void of hardness by other methods.

A robust mechanical and/or recirculating agitation system is essential during the production of this anticoagulant.

A portion of the NaOH can then be added carefully. The exothermic acid/base reaction brings about a 20° to 40° F. temperature rise and changes the appearance of the mixture from milky to clear. The remainder of the soft water is then added followed by the slow addition of $(NaPO_3)_6$ to the mixture at the point of maximum agitation.

If the $(NaPO_3)_6$ is added too rapidly, if it is added at a region of inadequate agitation, or if the mixing unit does not have adequate agitation capacity, it is likely that agglomeration of the hexametaphosphate will occur. If the $(NaPO_3)_6$ agglomerates, the time required for it to completely solubilize will increase significantly.

After the aqueous mixture is completely dissolved, the final portion of the sodium hydroxide is added to bring the pHoto the desired range. The exothermic reaction brought about by adding the final portion of the NaOH will cause a 15° to 20° F. temperature rise. It is believed that anticoagulant activity of the preparation will be optimal when the pH is adjusted to a value in the range between about 6.7 and about 6.8.

Other equivalent compounds may be substituted for the materials specifically identified in this disclosure. In addition, substitute materials known to those skilled in the art may be adapted to function in place of those specifically identified without departing from the teachings of this specification and the appended claims. Examples of some of the equivalent substitute constituents for the present anticoagulant preparation are described below:

Citric acid may be replaced by tartaric acid, succinic acid, fumaric acid, and by other polycarboxylic acids.

Sodium hexametaphosphate can be replaced by hexametaphosphate of other alkali metals, with potassium hexam-

etaphosphate being a substitute that is likely to be available commercially. Additional compounds that are deemed to be equivalent substitutes for the purposes of this disclosure include alkali metal glassy phosphates and alkali metal polyphosphates; examples of such compounds include sodium pyrophosphate ($Na_4P_2O_7$) and potassium pyrophosphate ($K_4P_2O_7$). Alkali metal tripolyphosphate, likewise, can be substituted in this anticoagulant preparation for the preferred sodium hexametaphosphate.

Some, and perhaps all, of the substitutes identified above will exhibit less than optimal performance for various reasons. For example, replacing part or all of the citric acid and hexametaphosphate with other acids and polyphosphates would reduce the efficacy of the product because both the solubility of the chelating agent and the chelating strength would be somewhat diminished.

Having described the preparation of the product in the foregoing paragraphs, it is to be appreciated that the anticoagulant can be packaged in pails, drums, tanks, and other containers adapted for goods of this type. At the destination meat packing facilities, water can be added to prepare a diluted anticoagulant working solution that can be sprayed onto the raw blood and used to rinse equipment and work surfaces. Some widely used existing commercial anticoagulant products may also be diluted. The anticoagulant of the present disclosure, diluted by an extent in the range between about ten percent (10%) and about thirty percent (30%) greater than the dilution of known products in the prior art, appears to perform at least as well as, or better than, existing products. The higher dilution factors that can be used with the product disclosed result in a reduction in the amount of effort that must be expended to handle this product, as much as ⅓ less, than the amount required to ship, store, transport, pump, mix, and use currently available anticoagulant products.

Changes and modifications in the specifically described embodiments can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

That which is claimed is:

1. An aqueous animal blood anticoagulant preparation consisting essentially of:

a. a water portion in the range of between 55.0% and 65.0% by weight,

b. an alkali metal EDTA portion in the range of between 0.5% and 3.0%,

c. an alkali metal hexametaphosphate portion in the range of between 17.0% and 24.0% by weight,

d. a citric acid portion in the range of between 5.0% and 9.0% by weight, and

e. a sodium hydroxide portion effective to bring the animal blood anticoagulant preparation to a pH in the range between pH 6.6 and pH 7.2.

2. An animal blood anticoagulant according to claim 1 wherein the alkali metal EDTA is tetrasodium EDTA.

3. An animal blood anticoagulant according to claim 1 wherein the alkali metal hexametaphosphate is sodium hexametaphosphate.

4. An animal blood anticoagulant according to claim 1 wherein the alkali metal hexametaphosphate is potassium hexametaphosphate.

5. An animal blood anticoagulant according to claim 2 wherein the alkali metal hexametaphosphate is potassium hexametaphosphate.

6. A method of making an aqueous animal blood anticoagulant preparation comprising the steps of:

a. adding an amount of water to a vessel that is about 20% to 30% by weight of the amount of anticoagulant being prepared,

b. dissolving an effective amount of citric acid in a range between 5.0% and 9.0% of the amount of anticoagulant being prepared to the water with vigorous agitation,

c. adding to the mixture, and dissolving with vigorous agitation, an effective amount of alkali metal EDTA in the range of between 0.5% and 3.0% by weight of the amount of anticoagulant being prepared,

d. carefully adding with agitation an amount of sodium hydroxide corresponding to about 3% to 4% by weight of the amount of anticoagulant being prepared,

e. adding the balance of the water with agitation to bring the total amount of water added to the mixture within the range of between about 55% and about 65% by weight of the amount of anticoagulant being prepared,

f. slowly adding to the vigorously agitated mixture, at the region of maximum agitation, an effective amount of alkali metal hexametaphosphate that is in the range of between about 17.0% and 24.0% by weight of the amount of anticoagulant being prepared,

g. carefully adding with agitation an effective amount of sodium hydroxide to adjust the pH to a value in the range of between pH 6.6 and pH 7.2 that provides optimal chelating activity.

7. The method of making an aqueous animal blood anticoagulant preparation defined in claim 6 wherein sodium hydroxide is added to adjust the pH to a value in the range of between 6.70 and 6.80.

8. The method of making an aqueous animal blood anticoagulant preparation defined in claim 6 wherein the alkali metal hexametaphosphate is sodium hexametaphosphate.

9. The method of making an aqueous animal blood anticoagulant preparation defined in claim 6 wherein the alkali metal hexametaphosphate is potassium hexametaphosphate.

10. The method of making an aqueous animal blood anticoagulant preparation defined in claim 6 wherein the water is soft water.

11. The method of making an aqueous animal blood anticoagulant preparation defined in claim 7 wherein the water is soft water.

12. The method of making an aqueous animal blood anticoagulant preparation defined in claim 8 wherein the water is soft water.

13. A composition made by combining:

a. an aqueous solution containing citric acid in the range of between 50% and 90%, by weight with,

b. tetrasodium EDTA in the range of 0.5% and 3.0% by weight,

c. alkali metal hexametaphosphate in the range of between 17% and 24% by weight, and

d. sodium hydroxide to adjust the pH to within the range of between pH 6.6 and pH 7.2.

14. The composition of claim 13 wherein the alkali metal hexametaphosphate is sodium hexametaphosphate.

15. The composition of claim 13 wherein the alkali metal hexametaphosphate is potassium hexametaphosphate.

16. A method of reducing the unwanted coagulation of animal blood in slaughterhouses comprised of the steps of:

a. suspending a freshly killed animal from a moving conveyor above a blood collection trough, the blood collection trough having an elongated axis directly below and parallel to the direction of conveyor movement,

b. draining the blood from the animal into the trough while the conveyor moves,

c. spraying an anticoagulant onto the surface of the trough and blood, the anticoagulant consisting essentially of;

i. an aqueous solution containing citric acid in the range of between about 0.4% and about 2% by weight with,

ii. tetrasodium EDTA in the range of between 0.5% and 2.0% by weight,

iii. alkali metal hexametaphosphate in the range of between 1% and 6% by weight, and

iv. sodium hydroxide to adjust the pH to within the range of between pH 6.6 and pH 7.2.

17. The method defined in claim 16 wherein the anticoagulant used for spraying is prepared from a concentrated solution that is diluted with soft water by a dilution factor in the range of between 4:1 and 20:1.

18. The method defined in claim 17 wherein the anticoagulant is prepared by metering concentrated anticoagulant into a diluent supply conduit, mixing in-line, followed by spraying the anticoagulant.

19. The method defined in claim 18, further comprised of the step of conveying the collected blood to a receptacle for storage.

* * * * *

US006221395B1

(12) **United States Patent**
Maggi et al.

(10) **Patent No.: US 6,221,395 B1**
(45) **Date of Patent: Apr. 24, 2001**

(54) **CONTROLLED RELEASE PHARMACEUTICAL TABLETS CONTAINING AN ACTIVE PRINCIPLE OF LOW WATER SOLUBILITY**

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(22) Filed: **Sep. 1, 1998**

(30) **Foreign Application Priority Data**

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(52) **U.S. Cl.** **424/475**; 424/474; 424/464; 424/468

(58) **Field of Search** 424/464, 468, 424/472, 474, 479, 480, 475

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Abstract of U.S. 5, 419,917, granted May 30, 1995, in the name of Chih-Ming Chen et al.

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(57) **ABSTRACT**

It is described a new method for the preparation of pharmaceutical tablets carrying poorly soluble in water principle; this method allows to obtain tablets with fast and/or slow release of the active principle. The peculiar feature is the fact that the poorly soluble in water active principle (es: nifedipine) is treated with a surfactant, during the granulation phase or whatever during the preparation process; the obtained product, subjected to a compression, produces pharmaceutical tablets which show high bioavailability of the carried active principle. This procedure can be used to prepare polymeric matrixes (with modified release), formed by tablets with one or more layers. The procedure of manufacture and the characteristics of the new finished tablet are described.

4 Claims, 1 Drawing Sheet

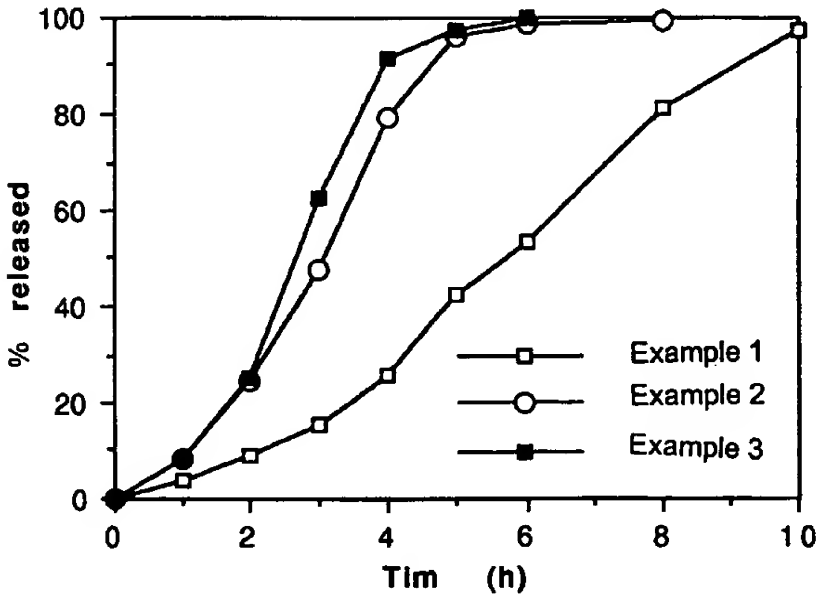


Fig. 1

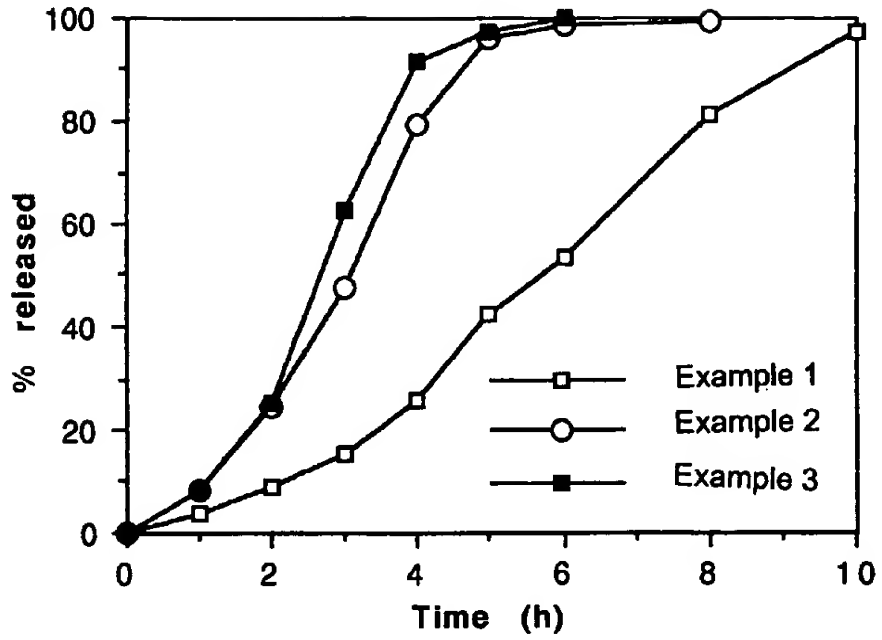
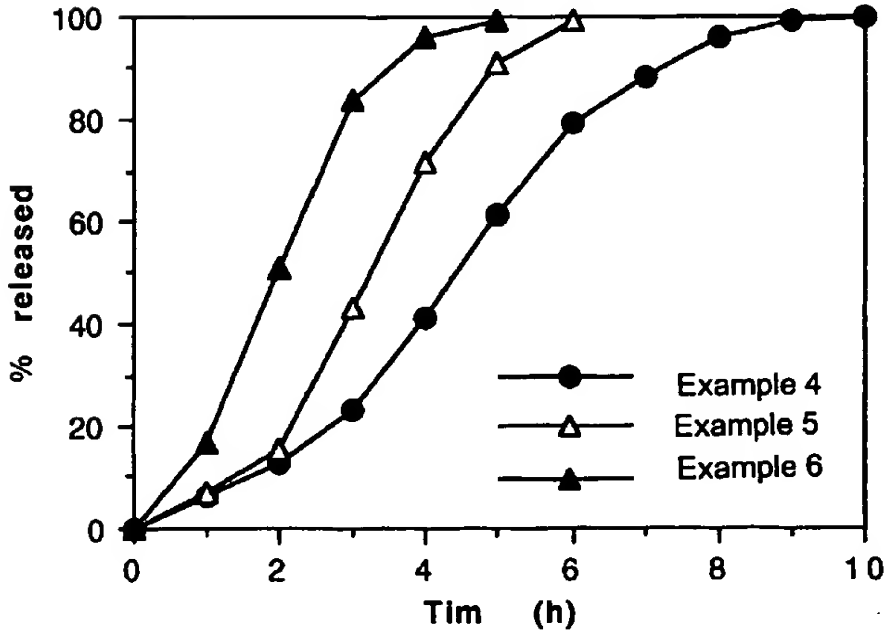

100
80
60
40
20
0
% released
0
2
4
6
8
10
Time (h)
Example 1
Example 2
Example 3

Fig. 2


100
80
60
40
20
0
% released
0
2
4
6
8
10
Tim (h)
Example 4
Example 5
Example 6

CONTROLLED RELEASE PHARMACEUTICAL TABLETS CONTAINING AN ACTIVE PRINCIPLE OF LOW WATER SOLUBILITY

FIELD OF THE INVENTION AND STATE OF THE ART

Biological active agents poorly soluble in water or in biological fluids have always represented remarkable problems of bioavailability when they are administered in pharmaceutical formulations for oral use.

This problem happens both in the case of preparation of fast release tablets and, mostly, in the case of tablets and/or whatever of therapeutic systems from which the active principle has to be released in an extended interval of time.

In fact, in the case of so-called "retard" tablets, the very low solubility of the drug produces a very changeable release speed of the active principle from the pharmaceutical formulation and, as consequence, a changeable and wrong absorption and, therefore, therapeutical effect.

Practically the dissolution speed of the active principle results as the restrictive factor of the absorption process and the followed therapeutic activity.

Many attempts have been carried out to modify the parameters which influence the dissolution speed of a biological active and poorly soluble agent.

The employment of micronized active agents, which show so wide superficial area, results widely utilized and well known by the expert of the art.

In fact, the process of dissolution of an active agent is regulated of the Noyes and Withney law which is usually expressed in the following form:

$$\frac{dc}{dt} = \frac{DS(Cs - C)}{h}$$

wherein

dc/dt=dissolution speed; it is the quantity of agent that dissolves in the unit of time

D=diffusion coefficient of the substance (depending from the molecular weight, from the viscosity of the medium, from the temperature, etc.)

h=thickness of the diffusion layer

S=total superficial area exposed to the medium of dissolution

Cs=concentration of the agent in the diffusion layer

C=concentration of the drug in solution in mass.

Procedures of micronization have been used to increase the dissolution speed of a lot of active principles like, for example: chloramphenicol palmitate, terfenadine, nitrofurantoine, naftazone, griseofulvine, even if, in this last case, an increase of the dissolution speed gives a great increase both of the activity but mostly of the toxicity of the product.

Also in the case of nifedipine, a drug widely used in the treatment of hypertension, it is known that a rapid effect of the drug can be obtained by using the micronized product, while by using the active principle in a greater granulometry or more precisely with a superficial area lower than 5 m^2/g it is obtained a retard in the dissolution speed and therefore a slower absorption speed and, consequently, a retarded therapeutical effect. This last procedure to obtain slow release pharmaceutical formulations has been claimed in German patents 2,209,526 and DE A1 3,033,991 even if the active agent with a precise granulometry and/or with a superficial area under the limits described in the quoted patent is particularly complex and not easily standardizable to obtain.

Different methods have been carried out to increase the dissolution speed of poorly soluble active agents like, for example, the transformation of the active substances from a crystalline to an amorphous state which represents, usually, an increase of the solubility and so of the dissolution speed too, for example the 1-acetoxy-ethyl-cefuroxime (axetylcefuroxime) (v. Gouda M. W. et al: Drug Develop. & Ind: Pharm. 3, 273, 1977).

To obtain similar results clathrates or inclusion complexes with polymers like polyvinylpyrrolidone, polyoxyethylenglycol, polyvinylalcohols, celluloses and derivatives have been prepared and in particular the complexes with cyclodextrins have provoked a lot of interest, even if it has to be underlined that these modifications involve a great increase of weight of the pharmaceutical formulation because at least a ratio of 1:1 molar between the drug and the polymer is usually utilized.

A wide series of possibilities to increase the dissolution speed of poorly active principles is described in the test "Technique of solubilization of drugs" of S. H. Yalkowsky-M. Dekker New York 1985.

A different technique to increase the dissolution speed of not very soluble active agent is claimed in the Italian patent no 1.188.165 (see application no 20474 A/85) and in the Italian patent no 1.246.188 and in the U.S. Pat. No. 5,476,654 in which it is used a procedure to load the poorly soluble drug on a support formed by hydrophylic swollen polymers or through a co-mixing and/or co-grinding process.

Nevertheless, all these methods allow to obtain an increase of the dissolution speed of an active principle but they don't guarantee a better bioavailability of the same, in particular, when this poorly soluble active principle, is carried out in a pharmaceutical formulation for oral use.

Like above mentioned, an increase of the dissolution speed of a poorly soluble drug is necessary for the preparation of fast-release tablets, but mostly for modified-release tablets, in order to allow that the absorption of the drug is not limited from the speed of its solubilization.

In the particular field of the controlled or modified release, the systems able to release the active principle at constant speed during time, namely systems that are usually defined with zero-release kinetics, are great important.

In fact, for example, in the case of hydrophilic matrixes, which form the class of the most utilized and diffused pharmaceutical formulations, the release of the drug shows at the beginning the fast release of a dose fraction ("burst effect"), phenomenon which has to be avoided because it can determine the outbreak of toxic effects linked to excessive absorption.

To avoid the "burst effect" different solutions have been proposed and adopted like to save a fraction of the matrix surface with a waterproof layer, at least for a determined interval of time, like described in the U.S. Pat. No. 4,839,177 and U.S. Pat. No. 5,422,123.

A different solution to the problem of the "burst effect" is the suggestion to add, in the formulation of the hydrophilic matrix, ionizable, pharmaceutically acceptable, compounds. This last solution is reported in the U.S. Pat. No. 5,419,917 in which it is described that the employment of polar substances in an hydrophilic matrix shows a great reduction of the dissolution speed of the active principle carried in the hydrophylic matrix.

DESCRIPTION OF THE INVENTION

Now we have unexpectedly found, and it is the object of the present industrial patent, that, the use of particular concentrations of surface-active agents in a hydrophilic matrix, allow to obtain an increase of the dissolution speed of a poorly soluble drug and, in this way, also an improvement of the absorption and bioavailability of the active principle carried in this matrix.

These systems of matrix release, composed by pharmaceutical tablets of one or more layers, one of which contains the active principle, can be produced by using precise productive and high industrial reproducible technologies. Moreover, we have, unexpectedly found that these systems do not determine "burst effect" and especially, they allow to eliminate the variability of the absorption caused by differences in the granulometry of the poorly soluble active principle.

In this way we have carried out and experimentally proved a new therapeutic system, with modified and controlled release, that solves the problem of the "burst effect" bound to the matrix systems. This system shows innovative advantages of safety and therapeutic efficacy, because the release of the active principle happens in a complete and reproducible way and the absorption results effective and high, like it is showed by the data relative to the plasma concentrations (C_{max}), obtained after the administration to the healthy volunteer, as it will be reported in the examples of the present patent.

Object of the present invention is a tablet of one or more layers one of which, at least, carries the active principle while the other one, or the others layers, have mostly the function of barrier with the purpose to modulate, for a determinable period of time, the release of the carried drug from the layer including the drug (For the geometry of the systems with more layers it refers back to what described in the above U.S. Pat. No. 5,422,123).

One of the characteristics of the tablet of the invention consists in the fact that in the preparation of the treated layer (or nucleus), beyond the active principle and a surface-active agent, also polymeric substances are utilized able to modulate (to slow down and/or to speed up) the release of the active principle.

As poorly very soluble in water substances (which show a solubility at 20° C. less then 50 mg/ml) many drugs can be used, including, in order to illustrate and not to limit: nifedipine, ricardipina, nitrendipine, nimodipine, niludipine, nilvadipine, nisoldipine, fenofibrate, naftazone, terfenadine.

These poorly soluble in water active substances are included in the treated layer (or nucleous) in a percentage from 9 to 80% of the weight, preferably from 20 to 60%.

The system is characterized by the fact that in the preparation of said nucleus or layer which containing the active principle, surfactant substances or substances with hydrophilic characteristics of acceptable pharmaceutical type, are used, selected from the group consisting of:

anionic surfactants

cationic surfactants

non-ionic surfactants

polyoxyethylenglycols (PEG) with molecular weight from 200 to 200,000

copolymers to polyoxyethylenic/polyoxypropylenic blocks (Poloxamer)

N, N', N", N"' tetra-(polyoxyethylen)(polyoxypropylen) diaminoethylene (Tetronic, Poloxamine)

dimethylpolysiloxane (Simethicone).

In order to illustrate and not to limit, the substances with surfactant properties, the following ones are reported: sodium lauryl sulphate, aluminium monostereate, sodium cetostearyl sulphate, magnesium and ammonium lauryl stearate, mono-, di-, triethanolamine laurylstearate, glycerylmonostearate, glycerylmonoleate, lauromacrogols (polyethoxylated laurylic alcohol), polysorbates of different pharmaceutical degree (they usually contain from 20 to 120 mols of C_2H_4O), esters of sorbitane with fatty acids, alkyldimethyl-(phenylmethyl) ammonium hydrochloride, cholesterol, bile acids and relative salts or esters or derivatives, lecithines, nonoxynoles or macrogolnonylphenylethers (polyethoxylated nonylphenols).

Said surfactants can be added to the active agent either with simple mixing or, in the case of a previously prepared granulated, using other components, too, like coadjuvants. These surfactants can be added, for example, to the binder solution, like it is well known in the prior art.

These hydrophyle or surfactants substances are included in the pharmaceutical formulation in a percentage from 1% to 40% of the weight of the treated layer, preferably from 2% to 30%.

As polymeric substances in the preparation of said layer (or nucleus) can be used, for example, reticulated polyvinylpyrrolidone, hydroxypropylmethylcellulose, reticulated sodium carboxymethyl-cellulose, carboxymethylstarch, potassium methacrylate-divinylbenzene copolymer, polyvinylalcohols, hydroxypropylcellulose at molecular weight from 2,000 to 4,000,000, carboxyvinylpolymers, glucanes, scleroglucanes, mannanes, galattomannanes, gellanes, xanthanes, alginic acid and derivatives, polyanhydrydes, polyaminoacids, poly-(methyl vinyl ethers/maleic anhydryde), carboxymethylcellulose and derivatives, ethylcellulose, methylcellulose and in general cellulosic derivatives, starchs, starch derivatives, alfa, beta, gamma cyclodextrins and in general dextrin derivatives.

These polymeric substances form from 3% to 90% of the weight of the layer (or nucleous), but preferably from 5% to 50%.

For all above polymers, many types characterized by different chemical and physical properties, solubility and gelling are present in the market, in particular, regarding the hydroxypropylmethylcellulose many types with different molecular weight (from 1,000 to 4,000,000) and different level of substitution can be used. Said types of hydroxypropylmethylcellulose show different characteristics because they are usually erodible and able to produce gels, by the way of the viscosity and the degree of substitution (D.S.) shown in the polymeric chain.

At least, usually in pharmaceutical technique excipients like: mannitol, lactose, sorbitol, xylitol, talc, stearic acid, sodium benzoate, magnesium stearate, colloidal silica and others like glyceryl monostearate, hydrogenated ricine oil, waxes, mono, bi-, trisubstituted glycerides, glycerilpalmitostearate, glyceryl behenate, cetylic alcohol can be used.

When it is desired to allow the penetration of water and/or aqueous fluids in the layer or nucleous, hydrophilic diluents are included like mannitol, lactose, starchs of different source, sorbitol, xylitol, or to carry in the formulation moistening substances and/or in general favouring the penetration of water in the compact. When it is desired to slow down the penetration of water and/or aqueous fluid in the treated layer or nucleus, hydropholic diluents are included like glyceryl monostearate, hydrogenated castor oil, waxes, mono-bi-trisubstituted glycerides. Moreover substances can be used like diluents, binders, lubricants, buffers, not adhesives, glydants, plasticizers and other substances, able to give to this layer the wanted characteristics like in the examples afterwards reported.

The pharmaceutical tablets of the invention have the advantage to release the carried active principle in a programmed way.

The system, in the simplest achievement, is a tablet with one or more layer at least one of which contains the active agent.

The formulation of the "barrier" layers includes polymeric substances and coadjuvants and plasticizer substances; when this tablet is of three layers called "barrier", they either can be similar one each other both for the composition and the thickness or they can be different.

The polymeric substances carried out in the different "barrier layers" are reported in the previous description of the nucleus or treated layer.

These polymeric substances occur in a percentage from 5% to 90% of the total weight of this layer and preferably from 50% to 90%.

Similarly, for the preparation of said layers, the coadjuvant substances previous described can be utilized.

It is possible to produce these systems with one or more layers, by using installations and equipments of widely consolidate use in pharmaceutical field and able to assure a safe and precise realization of the system with not much expensive cost (es: Elisabeth Hata).

Over these finished tablets, further polymeric coating material can be applied in order to cover the system, and to allow a protection for the tablet or a protection against light for the photosensitive active principle carried by this tablet or it can be a further slowing down in the beginning phase of the release.

Said coating can be soluble in an acid medium or permeable or it can be gastric resistant and enterosoluble, in order to allow the activation of the system only after the arrival of the tablet in the intestinal tract.

For the coating of these systems, the classical materials for the sugar coating or either natural and/or synthetic rubbers, like shellac, sandarac rubber, etc. or lypophylic material like natural waxes (white or yellow) or semisynthetic derivatives can be used.

Moreover film forming polymeric materials can be used, like: cellulose derivatives (hydroxypropylmethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose and their derivatives), acrylic and methacrylic copolymers of different molecular weight. In order to obtain the gastric resistance, many materials can be employed, like: zein, cellulose acetophthalate, cellulose acetopropionate, cellulose trimellitate, polyvinyl acetate phthalate, acrylic and methacrylic polymers and copolymers of different molecular weight and with a solubility that depends from different values of pH.

Said materials can be applied on the finished pharmaceutical formulation (tablet with one or more layers) through the classic method of film coating by using solutions in organic solvents or aqueous dispersions and working with a basin for atomization or in fluidized bed.

Said both gastric-soluble or gastric-resistant and enterosoluble materials can be employed in association with other retardant polymers and in association with other substances which have the function of plasticizers like: triethylcitrate, diethylphthalate, benzylbenzoate, dibutylsebacate, sorbitol, propylenglycol, diacetin, triacetin, dibutylphthalate, tributylacetate, castor oil, cetyl alcohol, cetylstearyl alcohol, fatty acids, polyoxyethylenglycols, usually selected from the group having a molecular weight from 200 to 200,000.

The coating layer can be applied, too, through the method of dry coating by using the above described materials, possibly previously granulated, like every expert of the field well knows.

The examples and the obtained results in the described experimental trials put better in evidence the characteristics and the functionalities of the new system. In any case, the innovation of the realization is characterized by the fact that the claimed therapeutic system can be obtained by using the usually productive technologies, that is the system is transferable in an industrial process.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the drug release in percent on the starting whole content, during the time, for the tablets according to Example 1, 2, and 3.

FIG. 2 shows a drug release in percent on the starting whole content, during the time, for the tablets according to Example 4, 5, and 6.

EXAMPLE 1

Preparation of a series of (5,000) tablets containing nifedipine 60 mg as active principle.

1-a-Composition of the first layer:

		% weight
Nifedipine (0.5 m^2/g)	60.0 mg	41.66
Lactose monohydrate (USP grade)	40.0 mg	27.77
Hydroxypropylmethylcellulose (Methocel K100 M, Colorcon, Orpington, UK)	20.0 mg	13.88
Polyvinylpyrrolidone (Plasdone K29-32, I.S.P., Wayne, NY, USA)	10.0 mg	6.94
Sodium laurylsulphate	10.0 mg	6.94
Magnesium stearate (C. Erba, Milano, I)	2.0 mg	1.4
Colloidal silica (Syloid 244, Grace GmbH, Worms, D)	2.0 mg	1.4
Total	144.0 mg	

1-b-Composition of the second layer:

Hydroxypropylmethylcellulose (Methocel E50 Premium, Colorcon, Orpington, UK)	36.65 mg
Lactose monohydrate (USP grade, C. Erba, Milano, I)	38.15 mg
Glyceryl behenate (Compritol 888ATO Gattefosse Saint Priest, F.)	18.80 mg
Polyvinylpyrrolidone (Plasdone K29-32 ISP Corp., Wayne, NY, USA)	5.00 mg
Yellow iron oxyde (Eingemann-Veronelli, Milano, I.)	0.10 mg
Magnesium stearate (USP grade, C. Erba, Milano, I.)	0.90 mg
Colloidal silica (Syloid 244, Grace GmbH, Worms, D)	0.40 mg
Total	100.0 mg

A quantity of granulate necessary to obtain 5,000 tablets with two layers is prepared.

The procedure of manufacture consists in the preparation of a wet granulate by using sigma mod. Erweka type K 5 mixer (Frankfurt a. M., D.), and by wetting the mixture of powders with an aqueous solution of polyvinylpyrrolidone at 10% (w/v) in which (in the case of the granulate of the first layer) the sodium laurylsulphate has been solubilized. The granulate is dried in a fluid bed apparatus (Aeromatic mod. Strea) and then added up by lubricants.

1-c-Preparation of systems with two layers (by compression).

The obtained granulates, like previously reported and according schemes well known by all the experts of the field, are carried out on the two charging hoppers of a rotary compression equipment which is suitable to produce two layer tablets (es. Elisabeth Hata). In particular in the first one the described granulate at 1-b point is carried; while in the second charging hopper the previously described granulate at 1-a point is carried.

The compression equipment, equipped with punches of 7.0 mm of diameter, is regulated in order to produce systems with two layers which are formed by a first layer of 144.0 mg containing the active principle (60 mg nifedipine) and by a second layer of 100 mg of barrier granulate.

1-e-Coating process of systems with two layers.

Composition of the coating:

Copolymer of the acrylic and metacrylic acid (Eudragit L30 D Rohm Pharma, D)	10.70 mg
Triethylcitrate (C, Erba, Milano, I)	1.60 mg
Iron oxyde	0.20 mg
Total	12.50 mg

The film forming process is done by using a coating apparatus (a basin) for rapid coating (Manesty Accela-Cota) by spraying, through an "air less" system an aqueous dispersion at the 30% of acrylic and metacrylic acid copolymer (Eudragit L 30 D) in which the triethylacetate is solubilized.

A temperature of about 40–50° C. is used for the entrance air, according to the known art, obtaining tablets completely covered by a uniform coating film of the previously reported polymeric materials.

EXAMPLE 2

Preparation of a series of (5,000) tablets containing Nifedipine 60 mg as active principle.

A two layers tablet is prepared with a composition exactly identical to that reported in the example 1 with only the substitution in the first layer formulation of the hydroxypropylmethylcellulose (Methocel K 100 M,) 20.0 mg with an identical quantity of hydroxypropylmethylcellulose (Methocel K 15 M). The second layer maintains identical composition.

EXAMPLE 3

Preparation of a series of (5,000) tablets containing Nifedipine 60 mg as active principle.

A two layers tablet is prepared with a composition exactly identical to that reported in the example 1 with only the substitution in the first layer formulation of the hydroxypropylmethylcellulose (Methocel K 100 M,) 20.0 mg with an identical quantity of hydroxypropylmethylcellulose (Methocel K 4 M). The second layer maintains identical composition.

f-Dissolution test

Example no 1, example no 2, example no 3:

In order to estimate the characteristics of the active principle release by the prepared and described systems in the example 1, 2, and 3, the apparatus 2 is used, paddle(USP XXII) by working at 100 r.p.m. and using as dissolution fluid 1 l of buffer solution at pH 6.8 formed by tris-hydroxymethylaminomethane 0.1M, which contains 1% of polysorbate 80. The release of the active principle is followed through the spectrophotometric UV determination by using an automatic system of sampling and reading (Beckman).

The results of the trials are reported in table 1.

TABLE 1

Time (h)	% released Example n°1	% released Example n°2	% released Example n°3
1	3.8	8.1	8.1
2	9.2	24.6	25.0
3	15.4	47.7	62.7
4	25.8	79.2	91.5
5	42.3	96.1	97.3
6	53.5	98.5	99.7
8	81.5	99.1	101.0
10	97.3		

From the analysis of table 1, it's evident that the utilization of hydroxypropylmethylcellulose of different molecular weight deeply modifies the release speed of the active principle. In particular in the example 1 hydroxypropylmethylcellulose is used at high molecular weight (Methocel K 100 M,) in the example 2 hydroxypropylmethylcellulose is employed at medium molecular weight (Methocel K 15 M) and in the example 3 at low molecular weight (Methocel K 4M) (see FIG. 1, too).

"In vivo" trials

In order to estimate the characteristics of bioavailability of the active principle carried out in the pharmaceutical formulation described in the example 1, a "cross over" experiment has been done with 12 healthy volunteers by using the medical speciality Procardia XL, as reference formulation, containing the same quantity of nifedipine.

In particular the following parameters has been determined:

C_{max}=maximum haematic concentration (peak) in ng/ml

T_{max}=time to achieve the peak

AUC(o–inf)=area under curve from 0 to infinity

The results are expressed as percentage in respect to the reference formulation.

C_{max}=91.0%

T_{max}=73.3%

AUC (o–inf)=90.9%

From the "in vivo" reported data, the pharmaceutical formulation described in the example 1 results bioequivalent in respect to the reference formulation, being AUC clearly over the 80%.

EXAMPLE 4

Preparation of a series of (5,000) tablets containing Nifedipine 60 mg as active principle.

A two layer tablet is prepared with a composition exactly identical to that reported in the example 1 with the only substitution in the first layer formulation of the quantity of sodium laurylsulphate used: instead of 10.0 mg 15.0 mg are employed. The method of production is the same too. The second layer maintains identical composition.

EXAMPLE 5

Preparation of a series of (5,000) tablets containing Nifedipine 60 mg as active principle.

A two layer tablet is prepared with a composition exactly identical to that reported in the example 1 with the only substitution in the first layer formulation of the quantity of sodium laurylsulphate used: instead of 10.0 mg, 20.0 mg are employed. The method of production is the same too. The second layer maintains identical composition.

EXAMPLE 6

Preparation of a series of (5,000) tablets containing Nifedipine 60 mg as active principle.

A two layer tablet is prepared with a composition exactly identical to that reported in the example 1 with the only substitution in the first layer formulation of the quantity of sodium laurylsulphate used: instead of 10.0 mg, 30.0 mg are employed. The method of production is the same too. The second layer maintains identical composition.

Dissolution test

Example no 4, Example no 5, Example no 6

In order to estimate the characteristic of the active agent release by the prepared and described systems in the example 4, 5, and 6, the apparatus 2 is used, paddle(USP XXII) by working at 100 r.p.m. and using as dissolution fluid 1 l of buffer solution at pH 6.8 composed by tris-hydroxymethylaminomethane 0.1M, which contains 1% of polysorbate 80. The release of the active agent is followed through the spettrophotometric UV determination by using a automatic system of sampling and reading (Beckman).

The results of the trials are reported in table 2.

TABLE 2

Time (h)	% release Example n°4	% release Example n°5	% release Example n°6
1	6.6	6.8	16.9
2	12.9	15.4	50.8
3	23.1	43.1	83.8
4	41.5	71.9	96.2
5	61.5	90.7	99.6
6	79.2	99.5	
7	88.5		
8	96.2		
9	99.2		
10	100.3		

From the analysis of table 2 it appears evident that the employment of growing quantities of sodium laurylsulphate in the preparation determines a great increase of the of release speed of the active principle from the pharmaceutical formulation (see FIG. **2**, too).

EXAMPLE 7

Preparation of a series of (5,000) tablets containing Nifedipine 60 mg as active principle.

7-a-Composition of the first layer:

		% weight
Nifedipine (0.5 m^2/g)	60.0 mg	41.66
Lactose monohydrate (USP grade)	30.0 mg	20.83
Hydroxypropylmethylcellulose (Methocel K100 M, Colorcon, Orpington, UK)	30.0 mg	20.83
Polyvinylpyrrolidone (Plasdone K29-32, I.S.P., Wayne, NY, USA)	10.0 mg	6.94
Polyaxycthylen glycol (Gattefosse Saint Priest, F)	10.0 mg	6.94
Magnesium stearate (C. Erba, Milano, I)	2.0 mg	1.4
Colloidal silica (Syloid 244, Grace GmbH, Worms, D)	2.0 mg	1.4
Total	144.0 mg	

7-b-Composition of the second layer:

		% weight
Hydroxypropylmethylcellulose (Methocel E50 Premium, Colorcon, Orpington, UK)	26.77 mg	38.34
Lactose monohydrate (USP grade, C. Erba, Milano, I)	26.77 mg	38.34
Glyceryl behenate (Compritol 888ATO Gattefosse Saint Priest, F.)	12.89 mg	18.41
Polyvinylpyrrolidone (Plasdone K29-32 ISP Corp., Wayne, NY, USA)	2.45 mg	3.5
Yellow iron oxyde (Eingemann-Veronelli, Milano, I.)	0.07 mg	0.1
Magnesium stearate (USP grade, C. Erba, Milano, I.)	0.70 mg	1
Colloidal silica(Syloid 244, Grace GmbH, Worms, D)	0.35 mg	0.5
Total	70.0 mg	

A quantity of granulate necessary to obtaining 5,000 tablets with two layers is prepared.

The procedure of manufacture consists in the preparation of a wet granulate by using sigma mod. Erweka type K 5 mixer (Frankfurt a. M., D.), and by wetting the mixture of powders with an aqueous solutions of polyvinylpyrrolidone at 10% (w/v) in which (in the case of the granulate of the first layer) the polyoxyethylenglycol has been solubilized. The granulate is dried in a fluid bed apparatus (Aeromatic mod. Strea) and then added up by lubricants.

7-c-Preparation of systems with two layers (by compression).

The obtained granulates, like previously reported and like schemes well known by all the experts of the field, are carried out on the two charging hoppers of a rotary compression equipment which is suitable to produce two layers tablets (es. Elisabeth Hata). In particular in the first one the described granulate at 7-b point is carried; while in the second charging hopper the previously described granulate at 7-a point is carried.

The compression-equipment, equipped with punches of 7.0 mm of diameter, is regulated in order to produce systems with two layers which are formed by a first layer of 144.0 mg including the active principle (like 60 mg nifedipine) and by a second layer of 70 mg of barrier granulate.

EXAMPLE 7

Dissolution test.

In order to estimate the characteristic of active principle release by the prepared and described system in the example 7, the apparatus 2 is used, paddle(USP XXII) by working a 100 r.p.m. and using as dissolution fluid 1 l of distilled water, which contains 1% of polysorbate 80. The active principle release is followed through the spectrophotometric UV determination by using an automatic system of sampling and reading (Beckman).

The results of the trials are reported in table 3

TABLE 3

Time (h)	% released Example n°7
1	2.2
2	5.0
4	12.0
6	21.4
8	31.2
10	40.4
12	49.5
16	67.4
20	84.6
24	98.8

"In vivo" trials

In order to estimate the characteristics of bioavailability of the active principle carried in the pharmaceutical formulation described in the example 7, a "cross over" experiment has been done with 12 healthy volunteers by using the medical speciality Procardia XL, as reference formulation, containing the same quantity of nifedipine.

In particular the following parameters has been determined:

C_{max} and AUC

The results are referred as percentage compared to the reference formulation:

C_{max}=123.3%

AUC (0–inf)=90.8%

From the "in vivo" reported data, the pharmaceutical formulation described in the example 7 results bioequivalent in respect to the reference formulation, being AUC clearly over the 80%.

What is claimed is:

1. A pharmaceutical tablet for oral administration, able to release under controlled speed, nifedipine, said tablet, consisting essentially of at least one first layer obtained by

compression of a mixture of ingredients in the form of a powder or granulate, said first layer comprising 9% to 80% by weight of nifedipine based on the total weight of said first layer, said first layer further comprising sodium lauryl sulfate at a ratio of 6:1 by weight of the nifedipine and 3% to 90% of hydroxypropylmethylcellulose having a viscosity of 100,000 cps; one or more barrier layers which comprise from 5% to 90% of a component selected from the group consisting of pharmaceutically acceptable and biocompatible polymeric substances, based on the total weight of said barrier layer or layers having either the properties to swell or to form gels or slowly erode in contact with water or aqueous fluids and which control the release of the active principle, and a coating layer which completely covers said at least one first layer and said barrier layer, said coating layer comprising a copolymer which comprises acrylic and methacrylic acid.

2. A pharmaceutical tablet for oral administration, able to release under controlled speed, an active principle, having a water solubility at 20° C. which is lower than 50 mg/ml, said tablet consisting essentially of at least one first layer obtained by compression of a mixture of ingredients in the form of a powder or granulate, said first layer comprising 9% to 80% by weight of the active principle, based on the total weight of said first layer, said first layer further comprising sodium lauryl sulfate at a ratio of 6:1 by weight of the active principle and 3% to 90% of hydroxypropylmethylcellulose having a viscosity of 100,000 cps; one or more barrier layers which comprise from 5% to 90% of a component selected from the group consisting of pharmaceutically acceptable and biocompatible polymeric substances, based on the total weight of said barrier layer or layers having either the properties to swell or to form gels or slowly erode in contact with water or aqueous fluids and which control the release of the active principle, and a coating layer which completely covers at least one first layer and said barrier layer, said coating layer comprising a copolymer which comprises acrylic and methacrylic acid.

3. The pharmaceutical tablet of claim 2, wherein the active principle is selected from the group consisting of nifedipine, nicardipine, nitrendipine, nimodipine, niludipine, nilvadipine, nisoldipine, fenofibrate, naftazone, and terfenadine.

4. A pharmaceutical tablet for oral administration, able to release under controlled speed, an active principle selected from the group consisting of nifedipine, nicardipine, nitrendipine, nimodipine, niludipine, nilvadipine, nisoldipine, fenofibrate, naftazone, and terfenadine, said tablet consisting essentially of at least one first layer obtained by compression of a mixture of ingredients in the form of a powder or granulate, said first layer comprising 9% to 80% by weight of the active principle based on the total weight of said first layer, said first layer further comprising sodium lauryl sulfate at a ratio of 6:1 by weight of the active principle and 3% to 90% of hydroxypropylmethylcellulose having a viscosity of 100,000 cps; one or more barrier layers which comprise from 5% to 90% of a component selected from the group consisting of pharmaceutically acceptable and biocompatible polymeric substances, based on the total weight of said barrier layer or layers having either the properties to swell or to form gels or slowly erode in contact with water or aqueous fluids and which control the release of the active principle, and a coating layer which completely covers at least one first layer and said barrier layer, said coating layer comprising a copolymer which comprises acrylic and methacrylic acid.

* * * * *

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United States Patent [19]

Lagrange et al.

[11] Patent Number: 5,776,497

[45] Date of Patent: Jul. 7, 1998

[54] **PRODUCT BASED ON INORGANIC OR ORGANIC PARTICLES BEARING AN INDOLINE-BASED PRODUCT**

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[21] Appl. No.: **478,138**

[22] Filed: **Jun. 7, 1995**

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[30] **Foreign Application Priority Data**

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[52] **U.S. Cl.** **424/489**; 424/401; 424/63; 424/64; 424/707; 548/483; 548/484; 548/485; 548/490

[58] **Field of Search** 424/401, 63, 64, 424/70.7, 489; 548/483, 484, 485, 490

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Primary Examiner—Jyothsan Venkat

Attorney, Agent, or Firm—Jacobson, Price, Holman & Stern, PLLC

[57] **ABSTRACT**

A mineral or organic particle-based product comprising an indoline product, a method for preparing same, and the use thereof in cosmetics. The product is a powder consisting of mineral or organic particles smaller than 200 microns, and comprises, in and/or on the particles, an indoline product obtained by oxidative polymerization using at least one indoline.

21 Claims, No Drawings

PRODUCT BASED ON INORGANIC OR ORGANIC PARTICLES BEARING AN INDOLINE-BASED PRODUCT

This is a divisional application of Ser. No. 08/117,206, filed Dec. 29, 1993 now U.S. Pat. No. 5,496,543 which claims the benefit of PCT/FR93/00031 filed Jan. 13, 1993.

The present invention relates to a product in the form of inorganic or organic particles containing an indoline-based product in or on the particles to a process for preparing it and to its use in cosmetics, in particular for making up the exoskeleton and/or the skin and protecting the human epidermis against UV radiation.

Pigments based on metal compounds, such as, for example, black and brown iron oxides, are generally used in makeup compositions for the skin and the exoskeleton. These pigments are not, however, completely harmless, and pigments capable of presenting fewer problems in their cosmetic application are consequently sought.

It is also desired, on some occasions, to be able to impart to the hair a coloration which can, if necessary, be removed very rapidly.

The Applicant has just discovered that it was possible to prepare in vitro a product in the form of a powder composed of inorganic or organic particles containing, in and/or on the particles, one or more indoline-based products resulting from the oxidative polymerization of at least one compound of the indoline family defined below.

He discovered that the use of this product in particle form was especially advantageous in as much as these particles, when introduced into a cosmetically acceptable medium, distribute well in then composition, which spreads readily on the exoskeleton or the skin and displays considerable covering power.

He also found that the product thereby obtained possessed an especially advantageous coefficient of absorption of ultraviolet radiation.

Lastly, the presence of indoline-based product, resulting from the oxidative polymerization of at least one indoline compound, makes it possible to obtain colorations of the final product which are especially advantageous in their use in cosmetics, inasmuch as the colorations capable of being obtained with the pigments which were available and compatible with cosmetic application were relatively limited. The colorations thereby obtained are especially stable to light.

The term "indoline-based product" or "indoline-based polymer" will be used hereinafter to denote the product obtained by oxidation of at least one indoline defined below.

The subject of the present invention is hence a powder consisting of inorganic or organic particles containing an indoline-based product in and/or on the particles.

Another subject of the invention consists of the preparation of such a powder.

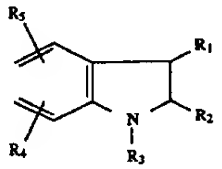
The subject of the invention is also the cosmetic application of such powders, in particular in products for making up the skin and the exoskeleton and for protecting the human epidermis against UV radiation.

The term "exoskeleton" according to the invention will be used to denote the hair, hairs such as eyelashes and eyebrows and the nails.

Other subjects of the invention will become apparent on reading the description and examples which follow.

The product according to the invention is essentially characterized in that it takes the form of a powder consisting of inorganic or organic particles whose largest dimension is less than 200 microns and which contains, in and/or on the particles, a synthetic indoline-based product formed in situ by oxidative polymerization of at least one indoline.

The indoline-based product results from the oxidation of at least one compound of the indoline family, corresponding to the formula:



(I)

in which:

- R_1 and R_3 represent, independently of one another, a hydrogen atom or a C_1–C_4 alkyl group;
- R_2 represents a hydrogen atom, a C_1–C_4 alkyl group or a carboxyl or (C_1–C_4 alkoxy)carbonyl group;
- R_4 denotes a hydrogen atom or a C_1–C_4 alkyl, hydroxyl, (C_1–C_4 alkoxy), amino or C_1–C_{10} alkylamino or halogen group;
- R_5 denotes a hydrogen atom or a hydroxyl, C_1–C_4 alkoxy or amino group;
- at least one of the radicals R_4 or R_5 denoting a hydroxyl, alkoxy or amino group; with the proviso that when R_5 denotes an amino group, R_4 cannot denote an alkylamino radical;
- R_4 and R_5 can also form a C_1–C_2 alkylenedioxy ring, and are at positions 5 and 6;

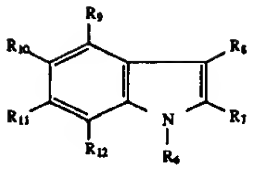
as well as the corresponding salts.

Among the compounds corresponding to the formula (I), the preferred compounds used according to the invention are chosen from 5,6-dihydroxyindoline, 6-hydroxy indoline, 5,6-methylenedioxyindoline, 7-methoxy-6-hydroxy indoline, 6,7-dihydroxyindoline, 5-hydroxy-4-methoxy indoline, 4,5-dihydroxyindoline, 5-methoxy-6-hydroxy indoline, 4-hydroxy-5-methoxyindoline, 5-hydroxy-6-methoxy indoline, 4,7-dihydroxyindoline, 6-aminoindoline, N-ethyl-4-hydroxyindoline, 1-ethyl-6-amino-indoline, 5,6-diaminoindoline, 1-methyl-6-aminoindoline, 2-methyl-6-aminoindoline, 3-methyl-6-aminoindoline, 2methyl 5,6-diaminoindoline, 5-chloro-7-aminoindoline, 3-methyl-5,7-diaminoindoline, 5,7-diaminoindoline, 2methyl-5,7-diaminoindoline, 7-aminoindoline, 2-methyl-7-amino indoline, 4-aminoindoline, 4-amino-6-chloroindoline, 4-amino-6-iodoindoline, 4-amino-5-bromoindoline, 4-amino-5-hydroxyindoline, 4-amino-7-hydroxyindoline, 4-amino-5-methoxyindoline, 4-amino- 7-methoxyindoline, 5-amino indoline, 2,3-dimethyl-5-aminoindoline, 1-methyl-5-aminoindoline, 2-methyl-5-aminoindoline, 5-[N-(1-methyl hexyl)amino]indoline, 5,6-dimethoxyindoline and 5,6-dihydroxy-2-carboxyindoline.

The salts of the abovementioned compounds are cosmetically acceptable salts, chosen especially from the hydrochlorides, hydrobromides, sulfates and methanesulfonates. The hydrobromides of the above compounds are especially preferred.

The indoline-based products can also result from the cooxidation of at least one defined indoline and at least one indole derivative. The latter may be chosen from mono- and dihydroxyindoles or aminoindoles as are described, more especially, in Patent EP-A-239,826 and Patent Applications EP-A-425,345 and GB-A-2,224,754.

These indoles correspond, more especially, to the formula:



(II)

in which:

R_6 and R_8 denote, independently of one another, a hydrogen atom or a C_1–C_4 alkyl group;

R_7 represents a hydrogen atom, a C_1–C_4 alkyl group, a carboxyl group or a (C_1–C_4 alkoxy)carbonyl group;

R_9 and R_{12} denote, independently of one another, a hydrogen atom, a hydroxyl group or a C_1–C_4 alkyl, amino, (C_1–C_4 alkoxy), (C_2–C_4 acyl)oxy or (C_2–C_4 acyl)amino group;

R_{10} denotes hydrogen or a hydroxyl, (C_1–C_4 alkoxy), (C_1–C_4 alkyl), halogen, amino, (C_2–C_{14} acyl)oxy, (C_2–C_4 acyl)amino or trimethylsilyloxy group;

R_{11} denotes hydrogen or a hydroxyl, (C_1–C_4 alkoxy), amino, (C_2–C_4 acyl)oxy, (C_2–C_4 acyl)amino, trimethylsilyloxy or hydroxy(C_2–C_4 alkyl)amino group;

R_{10} and R_{11}, together with the carbon atoms to which they are attached, can form a methylenedioxy ring optionally substituted with a C_1–C_4 alkyl or C_1–C_4 alkoxy group or a carbonyldioxy ring;

at least one of the groups R_9 to R_{12} represents a group OZ or NHR, not more than one of the groups R_9 to R_{12} denoting NHR;

and not more than two of the groups R_9 to R_{12} denote OZ, in the case where Z denotes hydrogen, these groups are at positions 5 and 6;

and at least one of the groups R_9 to R_{12} represents hydrogen, in the case where only one of these groups denotes hydrogen, only one group from among R_9 to R_{12} then denotes NHR or OZ and the other groups denote C_1–C_4 alkyl; R in NHR denoting a hydrogen atom or a C_2–C_4 acyl or C_2–C_4 hydroxyalkyl group, and Z in OZ denoting a hydrogen atom or a C_2–C_{14} acyl, C_1–C_4 alkyl or trimethylsilyl group; and the corresponding salts.

The indoles are chosen from 4-hydroxyindole, 5-hydroxyindole, 6-hydroxyindole, 7-hydroxyindole, 4-hydroxy-5-methoxyindole, 4-hydroxy-5-ethoxyindole, 2-carboxy 5-hydroxyindole, 5-hydroxy-6-methoxyindole, 6-hydroxy-7-methoxyindole, 5-methoxy-6-hydroxyindole, 5,6-dihydroxyindole, N-methyl-5,6-dihydroxyindole, 2-methyl-5,6-dihydroxyindole, 3-methyl-5,6-dihydroxyindole, 2,3-dimethyl-5,6-dihydroxy-indole, 2-carboxy-5,6-dihydroxyindole, 4-hydroxy-5-methylindole, 2-carboxy-6-hydroxyindole, 6-hydroxy-N-methylindole, 2-ethoxycarbonyl-5,6-dihydroxyindole, 4-hydroxy-7-methoxy-2,3-dimethylindole, 4-hydroxy-5-ethoxy-N-methylindole, 6-hydroxy-5-methoxy-2-methylindole, 6-hydroxy-5-methoxy-2,3-dimethylindole, 6-hydroxy-2-ethoxycarbonyl-indole, 7-hydroxy-3-methoxyindole, 5-hydroxy-6-methoxy-2,3-dimethylindole, 5-hydroxy-3-methylindole, 5-acetoxy-6hydroxyindole, 5-hydroxy-2-ethoxycarbonylindole, 6-hydroxy-2-carboxy-5-methylindole, 6-hydroxy-2-ethoxycarbonyl-5-methoxyindole, 6-[N-(β-hydroxyethyl)amino]indole, 4-aminoindole, 5-aminoindole, 6-aminoindole, 7-aminoindole, N-methyl-6-(β-hydroxyethylamino)indole, 6-amino-2,3-dimethylindole, 6-amino-2,3,4,5-tetramethylindole, 6-amino-2,3,4-trimethylindole, 6-amino-2,3,5-trimethylindole, 6-amino-2,3,6-trimethylindole, 5,6-diacetoxyindole, 5-methoxy-6-acetoxyindole and 5,6-dimethoxyindole.

In the cooxidation, it is possible to use up to 50 mol % of indole derivatives relative to the total number of moles of derivatives to be oxidized.

The particles used according to the invention are inorganic particles in lamellar or non-lamellar form or lamellar or non-lamellar organic particles, which are colored or uncolored. These particles have an average particle size of between 0.01 and 200 microns.

The non-lamellar inorganic particles which can be used according to the invention are inert inorganic particles having a particle size of less than 20 microns, and preferably less than 10 microns, and more especially less than or in the region of 5 microns.

Such particles are, in particular, chosen from calcium carbonate, silica or titanium oxide particles having the particle size defined above.

The lamellar particles used according to the invention are inorganic or organic particles which take the form of lamellae, where appropriate stratified.

These lamellae are characterized by a thickness which is smaller than the largest dimension. Preferably, the ratio between the largest dimension and the thickness is between 2 and 100. The largest dimension is generally less than 50 microns.

The particles of lamellar structure are chosen especially from the following products: L-lauroyllysine such as the product sold under the name AMIHOPE L.L by the company AJINOMOTO; microparticles of ceramic, which are optionally coated with zirconium powder, such as the products sold under the names TORAYCERAM ZP 550 and ZP 4000 by the company TORAY; lamellar titanium dioxide such as the products sold under the names LUXELEN SILK D and LUXELEN SS by the company SUMITOMO, lamellar talc, boron nitride such as the products sold under the names Boron Nitride SF or SHP by the companies WACKER and KAWASAKI; lamellar mica such at the product sold under the name MICA CONCORD 1000 by the company SCIAMA; bismuth oxychloride such as the product sold under the name PEARL GLO by the company MALLINCKRODT; and transparent red iron oxide such as the product sold under the name CAPPOXYT 4435 B by the company CAPPELLE.

The size of the particles of lamellar structure used according to the invention is preferably less than 50 microns, and especially less than 25 microns. Their size is generally greater than 0.5 micron. It is, in particular, between 1 and 20 microns. These particles are generally greater than 0.01 micron in thickness. As stated above, these lamellar particles can take the form of a stratified structure.

It is also possible to use colored particles according to the invention. They are colored, non-white inorganic particles consisting of metal salts which are insoluble in the cosmetic medium and usable in cosmetics, referenced in the Color Index under the section "Inorganic Coloring Matters" and which bear the numbers 77000 to 77947, excluding the white pigments. These colored inorganic particles can consist of a single pigment or a mixture of pigments. They can also take the form of nacreous or interference pigments.

The colored inorganic particles are preferably chosen from iron oxides, ultramarine blue (which is a complex sulfosilicate), chromium oxides, manganese violet (which is an ammonium manganese pyrophosphate) and Prussian blue (which is an iron ferricyanide).

The size of the particles of the colored powder, using colored inorganic particles containing the indoline-based product, depends on the size of the colored particles used, and can vary within wide limits ranging from 0.01 to 150 microns.

Thus, when the starting colored inorganic particle is a nacreous or interference pigment, the size of the powder particles according to the invention varies between 10 and 150 microns.

In contrast, when the colored inorganic particle is a metal salt (iron or chromium oxides, iron or manganese salts), the size of the powder particles according to the invention is generally between 0.01 and 5 microns.

The non-lamellar organic particles used according to the invention are fine particles of polymers. The product obtained with these organic particles according to the invention is characterized in that it consists of polymer particles having a particle size of less than 100 microns and which contain, at the surface and/or in the polymer network, an indoline-based product resulting from the oxidative polymerization of at least one compound of the indoline family corresponding to the formula (I) defined above and, where appropriate, from a cooxidation with an indole compound.

The size of these organic particles is generally greater than 0.01 micron, and preferably between 0.01 and 50 microns, and especially between 0.1 and 20 microns. They are preferably spherical.

The polymers which can be used according to the invention are polymers which are essentially insoluble in the reaction medium, and are chosen from natural or synthetic, organic or inorganic polymers comprising a crystalline or amorphous crosslinked network and having a molecular weight of between 5000 and 5.000.000.

The essentially insoluble character of the polymer is justified on essentially economic grounds, inasmuch as the indoline-based product must bind to a solid particulate carrier in order to form the product according to the invention.

The solubility of the polymers in the reaction medium should preferably not exceed 10%.

The organic or synthetic polymers are chosen especially from polymers derived from keratin, from chitin or from cellulose and polyamides, or homo- or copolymers resulting from the polymerization of aliphatic or aromatic mono- or polyethylenic monomers comprising a crystalline or amorphous crosslinked network.

The polymers derived from keratin are chosen especially from animal or human keratins originating, for example, from materials chosen from hair, wool, skin, hairs, silks, feathers, scales and more especially hooves, horn or alternatively silk fibroin.

These materials are preferably washed and/or degreased, and then reduced to particles.

Other polymers derived from keratin are chemically modified keratins having a molecular weight of between 10.000 and 250.000, and especially the partially hydrolyzed keratin (or keratin hydrolysate) obtained from skins which are rich in sulfur-containing products and have a molecular weight of between 50.000 and 200.000. This hydrolysate is preferably obtained by moderate alkaline hydrolysis.

Products of this type are, for example, sold under the name KERASOL by the company CRODA.

Other modified keratins are sulfonic keratins of molecular weight between 10.000 and 100.000, obtained from goose or chicken feathers or, still more advantageously, from hooves or horn.

This keratin is obtained by oxidation of all or part of the disulfide bonds of the cystine groups of keratin to cysteic acid groups: SO_3H, the oxidation being advantageously performed in an acid medium such as formic acid, by means of an oxidizing agent such as hydrogen peroxide.

The polymers derived from chitin consist of chitin which is a natural polymer, the largest source of which is in the shell of shellfish such as crabs, lobsters, crayfish, and the like. The chitin is prepared according to a process described, in particular, in the work of R. A. A. MUZZARELLI "CHITIN", published by PERGAMON PRESS OXFORD, 1977, pages 89–100 and 207–217. It is also possible to use its deacetylated derivative known by the name of chitosan, obtained by hydrolysis of the acetyl groups of chitin.

Chitosan as available commercially is partially acetylated and contains 70 to 90% by weight of chitosan.

It is also possible to use it in the form of its insoluble salts such as the sulfates and phosphates. Products of this type are sold, for example, under the name KYTEX by the company HERCULES.

The cellulose polymers are chosen more especially from microcrystalline celluloses such as the products sold under the name AVICEL by the company FMC.

Among synthetic polymers, very special mention may be made of polyethylene, polypropylene, polystyrene, poly(methyl methacrylate) and crosslinked poly(methyl methacrylate) such as the product sold under the name MICROPEARL M 305 by the company SEPPIC. Other polymers are chosen especially from crosslinked poly-β-alanine, as described in French Patent 2.530.250 or else advantageously taking the form of microspheres possessing a very low size dispersity, 85% by weight having a particle size of between 28 and 46 microns. These poly-β-alanines are obtained according to a process which consists in polymerizing acrylamide in a t-butanol/toluene solvent mixture in ratios of between 1:24 and 10:1, and preferably between 1:6 and 6:1, at between 60 and 100° C. and preferably at about 80° C. in the presence of a polymerization initiator and of an octadecene/maleic anhydride copolymer as suspending agent, and then in subjecting the poly-β-alanine suspension obtained to a crosslinking using a dialdehyde such as glutaraldehyde.

The polymerization initiator is preferably sodium tert-butylate or potassium tert-butylate (0.1 to approximately 2 mol % relative to the acrylamide).

Glutaraldehyde is used in the form of an aqueous solution of between 20 and 25%, and in a proportion of between 1 and 15% by weight relative to the weight of starting acrylamide.

As polymers, it is also possible to use products known by the name of microsponges, such as styrene/divinylbenzene or methyl methacrylate/ethylene glycol dimethacrylate or vinyl stearate/divinylbenzene crosslinked polymers as described in Patents WO-88/01,164 and U.S. Pat. No. 4,690,825.

Such polymers consist essentially of beads of crosslinked polymers containing an internal pore network capable of retaining the indoline-based product.

Other polymers of this type are hollow microspheres of a copolymer of vinylidene chloride and acrylonitrile, sold under the name EXPANCEL by the company KEMA NORD; and porous microspheres of polyamide 12, polyamide 6 or copolyamide 6/12, sold under the name ORGASOL by the company ATO-CHIMIE. These microspheres preferably have a particle size of between 10 and 50 microns.

It is also possible to use silicone powders which are gums, resins and more especially organosiloxane elastomers.

The products according to the invention may be prepared according to a process which consists essentially in mixing in the air, and at a temperature which is preferably room temperature and can range up to 100° C. at least one indoline compound of formula (I) and the inorganic or organic particles described above, in a medium which is essentially a non-solvent for the particles.

The oxidation of the indoline compound of formula (I) may be performed in an aqueous or water/solvent medium, in the air, in the air in the presence of an alkaline agent and/or of a metal-based oxidation catalyst such as cupric ion.

If no oxidizing agent other than aerial oxygen is used, it is preferable to work at an alkaline pH, in which case the pigment forms gradually and binds to the surface of the particles and/or in the network or the pores of the particles.

These products may also be prepared by carrying out an immediate formation of the indoline-based product using an oxidizing agent such as hydrogen peroxide, peracids and persalts.

It is preferable to use periodic acid and its derivatives and water-soluble salts, organic peracids and their salts, permanganates and dichromates such as those of sodium or potassium, sodium hypochlorite, potassium ferricyanide, ammonium persulfate, alkali metal chlorites, silver oxide, ferric chloride, lead oxide, sodium nitrite and rare-earth salts such as those of cerium.

It is also possible to use organic oxidizing agents chosen from ortho- and para-benzoquinones, ortho- and para-benzoquinone monoamines and diimines, 1,2- and 1,4-naphthoquinones and 1,2- and 1,2-naphthoquinone mono- or diimines. The oxidation may also be performed by the addition of iodide and hydrogen peroxide, the iodide preferably being an alkali metal, alkaline-earth metal or ammonium iodide.

The preferred periodic acid salt is sodium periodate.

These oxidizing agents may be activated, where appropriate, with a pH-modifying agent.

For the products intended for cosmetic application, it is preferable to use hydrogen peroxide, periodic acid and its salts, potassium permanganate, sodium hypochlorite, ammonium persulfate, sodium nitrite and the iodide/hydrogen peroxide system as oxidizing agents.

The order of addition of the compounds participating in the preparation of the product in particle form, according to the invention, is of little importance provided that the oxidizing agent is incorporated last when the latter is used without a pH-modifying agent and, in the case of the iodide/hydrogen peroxide oxidizing system, either hydrogen peroxide or the iodide is introduced last.

It is also possible to carry out enzymatic oxidation, carrying out oxidation using an enzyme having oxidizing or peroxidizing activity, such as horseradish peroxidase, chloroperoxidase, milk peroxidase and cytochrome C peroxidase, or peroxidizing enzymes, in particular hemoglobin, methemoglobin, myoglobin and metmyoglobin. This enzymatic oxidation may also be performed in the presence of tyrosinase with aerial oxygen.

In the case where a pH-modifying agent is used to activate the oxidizing agent, it is preferable to add either the oxidizing agent or the pH-modifying agent last.

When a keratin hydrolysate is used as an organic particle, the pH of the medium can preferably be below 5 in order to avoid solubilization of the modified keratin.

When a sulfonic keratin is used, the medium is either essentially alcoholic, or aqueous, in which case the pH should be below 7.

When chitosan is used, the aqueous medium should preferably have a pH above 5.8.

The reaction medium used should be a medium which is essentially a non-solvent for the organic or inorganic particle in question. It preferably consists of water, and can optionally consist of a mixture of water and one or more solvents such as C_1–C_4 lower alcohols, for instance ethyl alcohol, propyl or isopropyl alcohol and tert-butyl alcohol, alkylene glycols such as ethylene glycol and propylene glycol, alkylene glycol alkyl ethers such as ethylene glycol monomethyl, monoethyl and monobutyl ethers and propylene glycol and dipropylene glycol monomethyl ethers, and methyl lactate.

The medium must, moreover, be able to solubilize the indoline compound and, where appropriate, the indole derivative or derivatives.

When the medium consists of a water/solvent(s) mixture, the solvent(s) is/are present in concentrations preferably of between 0.5 and 95% by weight relative to the total weight of the composition, and especially between 2 and 50% by weight, and preferably between 2 and 20% by weight.

Their nature is chosen and their proportion is adjusted in accordance with the criteria of solubility of the derivatives of the family of the indolines (I) defined above, and, where appropriate, of the indoles of formula (II) in the case of cooxidation, and with the criterion of insolubility of the inorganic or organic particles employed.

In the process according to the invention, it is preferable to use the compounds of the indoline family in weight proportions of between 0.1 and 10%, and preferably between 0.5 and 7%, by weight, the inorganic or organic filler representing 0.075 to 70% by weight, and preferably 4 to 50% by weight, relative to the weight of the reaction medium, the remainder of the mixture generally consisting of water or a water/solvent mixture.

The oxidizing agents are employed in amounts sufficient to oxidize the compound of the family of the indolines of the formula (I), and, where appropriate, to cooxidize the indoline compound of the formula (I) and the indole compound of the formula (II), to form the indoline-based product on or in the inorganic or organic particles.

When iodide ion is used to form the indoline-based product, it is preferably used in proportions of 0.07 to 4%, and especially between 0.7 and 3%, observing a ratio of indoline compound and, where appropriate, indole derivative to iodide ions of between 0.6 and 6.

The proportions are determined relative to the weight of the reaction medium. When it is desired to carry out a cooxidation of one or more indoline derivatives of the formula (I) with one or more indole derivatives of the formula (II), the procedure is the same, simply mixing before oxidation of the derivatives to be oxidized.

The product based on particles containing the indoline-based product in and/or on the particle, according to the invention, is used in cosmetics, more especially in compositions for making up the skin and/or the exoskeleton and for protecting the human epidermis.

The powder in the form of inorganic or organic particles and containing the indoline-based product as defined above may be added to conventional cosmetic carriers at a concentration of between 0.05 and 35%, and preferably between 0.5 and 20%, by weight relative to the total weight of the composition, to yield cosmetic compositions for protecting the human epidermis, makeup products such as those for making up the eyelashes, eyebrows, skin, hair or nails, for instance eyeshadows, blushers, lining products also known as eyeliners, mascaras for the eyelashes and eyebrows, and nail varnishes, or alternatively compositions for the temporary dyeing of hair. These cosmetic carriers are known per se.

The medium used in these various cosmetic compositions is a medium which is essentially a non-solvent for the inorganic or organic particles containing the indoline-based product.

The term "medium which is essentially a non-solvent" is used to denote a medium which dissolves less than 1% by

weight of the inorganic or organic particles containing the indoline-based product.

The compositions can take the form, in particular, of a lotion, thickened lotion, gel, cream, milk, powder or stick, and can optionally be packaged as an aerosol and take the form of a foam or spray.

When the compositions are used for making up the skin, hair, eyelashes and eyebrows, they can, in particular, take anhydrous or aqueous pasty or solid form, for example oil-in-water or water-in-oil emulsions or alternatively suspensions. These compositions have the advantage of being stable and of affording complete safety.

When the compositions are used for protecting the human epidermis against UV radiation, they constitute so-called "sun" compositions, and they can take the form of suspensions or dispersions in solvents or fats, or alternatively the form of emulsions such as creams and milks, ointments, gels, solid sticks or aerosol foams.

In all cases, when they are used in the form of emulsions, they can contain, in addition, surfactants which are well known in the prior art, such as anionic, nonionic, cationic or amphoteric surfactants.

The makeup compositions and the sun compositions can also contain fats, organic solvents, silicones, thickeners, demulcents, sunscreen agents, antifoams, hydrating agents, perfumes, preservatives, antioxidants, fillers, sequestering agents, treatment agents such as anionic, cationic, nonionic or amphoteric polymers or mixtures thereof, propellants and alkalinizing or acidifying agents.

The fats can consist of an oil or a wax or a mixture thereof, fatty acids, fatty alcohols, petroleum jelly, paraffin, lanolin, hydrogenated lanolin or acetylated lanolin.

The oils are chosen from animal, vegetable, mineral or synthetic oils, and in particular hydrogenated palm oil, hydrogenated castor oil, liquid paraffin, paraffin oil and Purcellin oil.

The waxes are chosen from animal, fossil, vegetable, mineral or synthetic waxes. Special mention may be made of beeswax, carnauba, candellila [sic], sugar cane and Japan waxes, ozokerites, montan wax, micro-crystalline waxes and paraffins.

When the compositions are used for coloring the nails, they take the form of products termed "nail varnishes" containing the powder according to the invention in dispersed form in a cosmetically acceptable solvent containing one or more resins, and ingredients customarily used in this type of product.

The compositions according to the invention can also contain, in addition to the inorganic or organic particles containing the indoline-based product as is defined above, other pigments which are generally used in cosmetics, in particular nacreous and/or pearlescent, colored or white pigments which make it possible to vary the colorations capable of being obtained or to increase the protection with respect to ultraviolet radiation. In the latter case, it is preferable to use nanopigments of metal oxides such as titanium, zinc, cerium or zirconium oxides, of average diameter less than 100 nm and preferably between 5 and 50 nm. The pigments may be coated or uncoated.

The coated pigments are pigments which have undergone one or more surface treatments of a chemical, electronic, mechanochemical and/or mechanical nature with compounds such as those described, for example, in COSMETICS & TOILETRIES, February 1990, Vol. 105, pages 53–64, such as amino acids, beeswax, fatty acids, fatty alcohols, anionic surfactants, lecithins, sodium, potassium, zinc, iron or aluminum salts of fatty acids, metal alkoxides (of titanium or aluminum), polyethylene, silicones, proteins (collagen, elastin), alkanolamines, silicon oxides, metal oxides or sodium hexametaphosphate.

The subject of the invention is also a process for the temporary dyeing of hair, making up the skin, eyelashes and eyebrows or nails and protecting the human epidermis against the deliterious effects of UV radiation, employing a powder based on inorganic or organic particles containing indoline-based products as defined above, this powder being applied directly or by means of cosmetic compositions as defined above.

The examples which follow are intended to illustrate the invention, no limitation, however, being implied thereby.

PREPARATION EXAMPLES

Example 1

7.3 g (0.031 mol) of 5,6-dihydroxyindoline hydrobromide are solubilized in 100 ml of 0.1% aqueous ammonia solution. 45 g of polyamide 12 powder, sold under the name ORGASOL 2002 D Natural COS by the company ATOCREM, are added to this mixture. This suspension is stirred for 15 minutes and then brought to 80° C. 10.5 ml of 3N sodium hydroxide are then added, and 28.6 g of hydrogen peroxide solution containing 2.3 g (0.067 mol) of hydrogen peroxide are added in the course of 30 minutes while the temperature is maintained at between 80° and 85° C. When the addition is complete, the temperature is maintained at 80° C. for 2 hours and the reaction medium is then cooled. The product is centrifuged and washed with water. After lyophilization, 42 g of gray-brown powder are obtained.

Example 2

7.3 g (0.031 mol) of 5,6-dihydroxyindoline hydrobromide are solubilized in 100 ml of 0.1% aqueous ammonia solution. 45 g of boron nitride, sold under the name SHP2 by the company KAWASAKI, are added to this mixture. This suspension is stirred for 15 minutes and then brought to 80° C. 10.5 ml of 3N sodium hydroxide are then added, and 28.6 g of hydrogen peroxide solution containing 2.3 g (0.067 mol) of hydrogen peroxide are added in the course of 30 minutes while the temperature is maintained at between 80° and 85° C. The procedure is thereafter as in Example 1. After lyophilization, 41 g of bluish-gray powder are obtained.

Example 3

7.3 g (0.031 mol) of 5,6-dihydroxyindoline hydrobromide are solubilized in 100 ml of 0.1% aqueous ammonia solution. 45 g of non-lamellar red iron oxide, which is a mixture of yellow iron oxide (CI77492) and brown iron oxide (CI77491), are added to this mixture. The procedure is thereafter as in Example 1. After lyophilization, 41 g of brown powder are obtained.

Example 4

7.3 g (0.031 mol) of 5,6-dihydroxyindoline hydrobromide are solubilized in 100 ml of 0.1% aqueous ammonia solution. 45 g of ultramarine blue (CI77007) are added to this mixture. The procedure is thereafter as in Example 1. After lyophilization, 49 g of dark blue powder are obtained.

Example 5

7.3 g (0.031 mol) of 5,6-dihydroxyindoline hydrobromide are solubilized in 100 ml of 0.1% aqueous ammonia solu-

tion. 45 g of hydrated chromium oxide (CI77289) are added to this mixture. The procedure is thereafter as in Example 1. After lyophilization, 41.5 g of bluish-green powder are obtained.

Example 6

7.3 g (0.031 mol) of 5.6-dihydroxyindoline hydrobromide are solubilized in 100 ml of 0.1% aqueous ammonia solution. 45 g of silica, sold under the name SILICA BEADS SB 150 by the company MAPRECOS, are added to this mixture. The procedure is thereafter as in Example 1. After lyophilization, 45.8 g of gray-brown powder are obtained.

Example 7

7.3 g (0.031 mol) of 5.6-dihydroxyindoline hydrobromide are solubilized in 100 ml of 0.1% aqueous ammonia solution. 45 g of titanium oxide, sold under the name F. F. HOMBITAN by the company SACHTLEBEN, are added to this mixture. The procedure is thereafter as in Example 1. After lyophilization, 48.5 g of bluish-gray powder are obtained.

Example 8

4.38 g (0.0188 mol) of 5.6-dihydroxyindoline hydrobromide and 2.19 g (0.0147 mol) of 5.6-dihydroxyindole are solubilized in 100 ml of 0.1% aqueous ammonia solution. 45 g of ultramarine blue (CI77007) are added to this mixture. This suspension is stirred for 15 minutes and then brought to 80° C. 6.7 ml of 3N sodium hydroxide are then added, and 28.6 g of hydrogen peroxide solution containing 2.3 g (0.067 mol) of hydrogen peroxide are added in the course of 30 minutes while the temperature is maintained at between 80° and 85° C. When the addition is complete, the temperature is maintained at 80° C. for 2 hours and the reaction medium is then cooled. The product is centrifuged and washed with water. After lyophilization, 49 g of dark blue powder are obtained.

Example 9

15.4 g (0.065 mol) of 5.6-dihydroxyindoline hydrobromide are solubilized in 190 ml of 0.1% aqueous ammonia solution and 10 ml of ethanol. 90 g of L-lauroyllysine are added to this mixture. This suspension is stirred for 15 minutes and then brought to 80° C. 6.6 ml of 3N sodium hydroxide are then added, and 15.1 g (0.13 mol) of hydrogen peroxide diluted in 45.3 g of water are added in the course of 30 minutes while the temperature is maintained at between 80° C. and 85° C. When the addition is complete, stirring is maintained and the temperature is maintained at 80° C. for approximately 2 hours and the reaction medium is then cooled. The product is centrifuged and washed with water. After lyophilization, 93 g of black powder are obtained.

Example 10

13.95 g (0.06 mol) of 5.6-dihydroxyindoline hydrobromide are solubilized in 410 ml of 0.1% aqueous ammonia solution. 262 g of poly-β-alanine and also 50 ml of water are added to this mixture. This suspension is stirred for approximately 2 hours and then brought to 80° C. 6 ml of 3N sodium hydroxide are then added and the temperature is maintained at 80° C. for approximately 30 minutes. 13.82 g (0.12 mol) of hydrogen peroxide diluted in 41.46 g of water are then added in the course of 30 minutes while the temperature is maintained at between 80° C. and 85° C. When the addition is complete, the temperature is maintained at 80° C. and stirring is maintained for approximately 2 hours and the reaction medium is then cooled. The product is filtered off and washed with water. After lyophilization, 93.20 g of dark brown powder are obtained.

Example 11

7.68 g (0.033 mol) of 5.6-dihydroxyindoline hydrobromide are solubilized in 195 ml of 0.1% aqueous ammonia solution. 95 g of bismuth oxychloride, sold under the name "PEARL GLOW UVR 1086" by the company MALLINCKRODT, are added to this mixture. 3.3 ml of 3N sodium hydroxide are then added. The mixture is stirred for approximately 15 minutes and the temperature is then brought to 80° C.

7.55 g (0.07 mol) of hydrogen peroxide diluted in 22.7 g of water are then added in the course of 30 minutes while the temperature is maintained at between 80° C. and 85° C. When the addition is complete, the temperature is maintained at 80° C and stirring is maintained for approximately 2 hours and the reaction medium is then cooled. The product is centrifuged and washed with water. After centrifugation, 95.37 g of brown powder are obtained.

Example 12

7.68 g (0.033 mol) of 5.6-dihydroxyindoline hydrobromide are solubilized in 195 ml of 0.1% aqueous ammonia solution. 95 g of powder consisting of an 83:17 mica/titanium mixture are added to this mixture. The procedure is thereafter as described in Example 11. After lyophilization, 88.21 g of metallic-gray powder are obtained.

Example 13

15.4 g (0.065 mol) of 5.6-dihydroxyindoline hydrobromide are solubilized in 195 ml of 0.1% aqueous ammonia solution. 90 g of microbeads of acrylic polymer, sold under the name POLYTRAP by DOW CORNING, and also 10 ml of ethanol are then added to this mixture. The procedure is thereafter as described in Example 9. After lyophilization, 91.5 g of white powder are obtained.

FORMULATION EXAMPLES

Example 1

A creamy mascara of the following formula is prepared:

Triethanolamine stearate	15.0 g
Beeswax	8.0 g
Paraffin	3.0 g
Colophony	2.0 g
Ozokerite	10.0 g
Propyl para-hydroxybenzoate	0.20 g
Methyl para-hydroxybenzoate	0.20 g
Gum arabic	0.50 g
Keratin hydrolysate	1.0 g
Black iron oxide	5.0 g
Colored powder of Preparation Example 4	10.0 g
Water q.s.	100 g

The procedure is as follows:

The waxes are melted. The pigments are incorporated. The aqueous phase containing the preservatives, gum and keratin hydrolysate is heated to the same temperature as the waxy phase. The two phases are mixed and stirred vigorously. A dark blue mascara cream is obtained.

Example 2

An eyelash makeup composition as follows is prepared:

C_8-C_{30} alkyl acrylate/acrylate crosslinked copolymer	0.10 g
Crosslinked polyvinylcarboxylic polymer sold under the name CARBOPOL 940 by the company GOODRICH	0.60 g
Triethanolamine	0.80 g
Glycerol	2.0 g
Preservative	0.2 g
Octamethylcyclosiloxane	25.0 g
Colored powder of Preparation Example 3	5.0 g
Black iron oxide	5.0 g
Water q.s	100 g

The polymers are dispersed in the heated state with the preservatives in the water to make a gel. The glycerol and triethanolamine are added. The pigments are dispersed in the silicone and added to the gel phase. A black shiny gelled emulsion for making up the eyelashes is obtained.

Example 3

A lipstick of the following composition is prepared:

2,6-Di-tert-butyl-p-cresol	0.16 g
Liquid lanolin	17.50 g
Microcrystalline wax	15.0 g
Triglycerides of caprylic and capric acids	11.0 g
Octylglyceryl behenate	11.0 g
Colored powder of Preparation Example 3	3.0 g
Titanium mica	6.0 g
Castor oil q.s.	100 g

A nacreous-brown lipstick is obtained.

Example 4

A blusher of the following composition is prepared:

Titanium dioxide	10.0 g
Titanium mica	10.0 g
DC Red 30	1.2 g
Propyl para-hydroxybenzoate	0.2 g
Liquid paraffin	6.0 g
2-Hydroxy-4-methoxybenzophenone sold under the name UVINUL M40 by the company BASF	0.5 g
Colored powder of Preparation Example 6	3.0 g
Talc q.s.	100 g

This beige-pink compacted blusher is applied with a brush.

Example 5

An eyeshadow of the following composition is prepared:

Polyamide powder	15.0 g
Cyclomethicone	9.0 g
Titanium mica	30.0 g
Colored powder of Preparation Example 5	7.0 g
Colored powder of Preparation Example 1	6.0 g
Talc q.s	100 g

This gray-green eyeshadow is applied with a brush or with a foam applicator.

Example 6

A compact face powder of the following composition is prepared:

Polyethylene powder	5.0 g
Colored powder of Preparation Example 3	6.0 g
Titanium dioxide	10.0 g
Mica	12.0 g
Isopropyl myristate	1.5 g
Liquid paraffin	1.5 g
Sorbitol	0.5 g
Colored powder of Preparation Example 7	3.0 g
Talc q.s.	100 g

This natural beige powder is applied with a powder puff or brush.

Example 7

A makeup foundation for the face of the following composition is prepared:

Glycerol stearate	2.2 g
Mixture of capric and caprylic acids and glycerol triester	15.0 g
Titanium oxide	10.53 g
Yellow iron oxide	0.83 g
Synthetic melanin pigment	0.14 g
Colored powder of Example 3	0.50 g
Methyl para-hydroxybenzoate	0.10 g
Propyl para-hydroxybenzoate	0.10 g
Preservative	0.3 g
2-Hydroxy-4-methoxybenzophenone	0.5 g
Octyl dimethyl-p-aminobenzoate	0.5 g
Magnesium aluminum silicate	1.0 g
Triethanolamine	1.0 g
Carboxymethylcellulose	0.16 g
Aluminum salt of the reaction product of octenylsuccinic anhydride and starch, sold under the name DRY FIO [sic] by the company NATIONAL STARCH	5.0 g
Cyclic polydimethylsiloxane sold under the name SILBIONE OIL 70045 by the company RHONE POULENC	10.0 g
Propylene glycol	2.0 g
Glycerol	3.0 g
Sodium lauroylsarcosinate	0.6 g
Stearic acid	2.2 g
Water q.s.	100 g

The makeup foundation obtained is natural beige.

Example 8

A creamy mascara of the following composition is prepared:

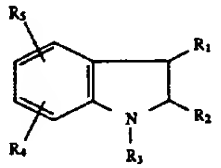
Triethanolamine stearate	15.0 g
Beeswax	8.0 g
Paraffin	3.0 g
Colophony	2.0 g
Ozokerite	10.0 g
Propyl para-hydroxybenzoate	0.20 g
Methyl para-hydroxybenzoate	0.20 g
Gum arabic	0.50 g
Keratin hydrolysate	1.0 g
Black iron oxide	5.0 g
Colored powder of Preparation Example 8	10.0 g
Water q.s.	100 g

The procedure is as follows:

The waxes are melted. The pigments are incorporated. The aqueous phase containing the preservatives, gum and keratin hydrolysate is heated to the same temperature as the waxy phase. The two phases are mixed and stirred vigorously. A dark blue mascara cream is obtained.

We claim:

1. Process for preparing a product in powder form, comprising mixing in air at a temperature between room temperature and 100° C. inorganic or organic particles of particle size of less than 200 microns, and an indoline compound of formula (I)



(I)

in which:

R_1 and R_3 represent, independently of one another, a hydrogen atom or a C_1–C_4 alkyl group;

R_2 represents a hydrogen atom, a C_1–C_4 alkyl group, a carboxyl group, a C_1–C_4 alkoxy carbonyl group, or an n-alkylamino radical;

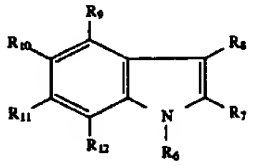
R_4 denotes a hydrogen atom, a C_1–C_4 alkyl group, a hydroxyl group, a C_1–C_4 alkoxy group, an amino group, a C_1–C_{10} alkylamino radical or a halogen atom;

R_5 denotes a hydrogen atom, a hydroxyl group, a C_1–C_4 alkoxy group or an amino group;

at least one of the radicals R_4 or R_5 denoting a hydroxyl, alkoxy or amino group; with the proviso that when R_5 denotes an amino group, R_4 cannot denote an alkylamino radical; or

R_4 and R_5 attached to the carbon atoms joined together form a C_1–C_2 alkylenedioxy ring, at positions 5 and 6;

as well as the cosmetically acceptable salts, or the indoline compound of formula (I) and at least one indole selected from the group consisting of monohydroxyindoles, dihydroxyindoles and aminoindoles, wherein the indoles have the formula;



(II)

in which:

R_6 and R_8 denote, independently of one another, a hydrogen atom or a C_1–C_4 alkyl group;

R_7 represents a hydrogen atom, a C_1–C_4 alkyl group, a carboxyl group or a (C_1–C_4 alkoxy) carbonyl group;

R_9 and R_{12} denote, indpendently of one another, a hydrogen atom, a hydroxyl group, a C_1–C_4 alkyl, amino, (C_1–C_4 alkoxy), (C_2–C_4 acyl)oxy or (C_2–C_4 acyl) amino group;

R_{10} denotes hydrogen, a hydroxyl, (C_1–C_4 alkoxy), (C_1–C_4 alkyl), halogen, amino, (C_2–C_{14} acyl)oxy, (C_2–C_4 acyl)amino or trimethylsilyloxy group;

R_{11} denotes hydrogen or a hydroxyl, (C_1–C_4 alkoxy), amino, (C_2–C_4 acyl)oxy, (C_2–C_4 acyl)amino, trimethylsilyloxy or hydroxy (C_2–C_4 alkyl)amino group;

R_{10} and R_{11}, together with the carbon atoms to which they are attached, can form a methylenedioxy ring optionally substituted with a C_1–C_4 alkyl, C_1–C_4 alkoxy group or a carbonyldioxy ring;

at least one of the group R_9 to R_{12} represents a group OZ or NHR, not more than one of the group R_9 to R_{12} denoting NRH and not more than two of the groups R_9 to R_{12} denoting OZ; at least one of the group R_9 to R_{12} represents hydrogen, in the case where only one of these groups denotes hydrogen only one group from R_9 to R_{12} then denotes NHR or OZ and the other group denote C_1–C_4 alkyl; R in NHR denotes a hydrogen atom or a C_2–C_4 acyl or C_2–C_4 hydroxyalkyl group; and Z in OZ denotes a hydrogen atom or a C_2–C_{14} acyl, C_1–C_4 alkyl or trimethylsilyl group, and in the case where Z denotes hydrogen, the OZ groups are at positions 5 and 6; and the corresponding salts, in a medium which is essentially a non-solvent for the inorganic or organic particles, and

forming the product in powder form by oxidation of the indoline of formula (I), or by cooxidation of the indoline of formula (I) and the at least one indole, on or in said particles or on and in said particles.

2. Process according to claim 1, wherein the oxidation is performed slowly in the air at an alkaline pH.

3. Process according to claim 1, wherein the oxidation is performed with aerial oxygen in the presence of a metal-based catalyst.

4. Process according to claim 1, wherein the oxidation is performed by adding oxidizing agents selected from the group consisting of hydrogen peroxide, periodic acid, salts of periodic acid, potassium permanganate, sodium hypochlorite, ammonium persulfate, the iodide/hydrogen peroxide combination, organic peracids, salts of organic peracids, alkali metal chlorites, potassium ferricyanide, silver oxide, lead oxide, ferric chloride, sodium nitrite, rare-earth salts, ortho-benzoquinones, para-benzoquinones, ortho-benzoquinone monoimines, ortho-benzoquinone diamines, para-benzoquinone monoimines, para-benzoquinone diimines, 1,2-naphthoquinones, 1,4-naphthoquinones, 1,2-naphthoquinone monoimines, 1,2-naphthoquinone diimines, 1,4-naphthoquinone monoimines, and 1,4-naphthoquinone diimines.

5. Process according to claim 4, wherein the oxidation is performed with hydrogen peroxide in an ammonical medium.

6. Process according to claim 1, wherein the oxidation is performed by the use of an alkali metal iodide, alkaline-earth metal iodide or ammonium iodide, preceded or followed by the addition of hydrogen peroxide.

7. Process according to claim 1, wherein the oxidation is an enzymatic oxidation.

8. Process according to claim 1, wherein the reaction medium is a medium which is essentially a non-solvent for the inorganic or organic particles and a solvent for the indoline of formula (I) and consists of water or a mixture of water and a solvent.

9. Process according to claim 8, wherein the solvent is selected from the group consisting of C_1–C_4 lower alcohols, alkylene glycols, alkylene glycol alkyl ethers and methyl lactate.

10. Process according to claim 1 wherein the indoline of formula (I) is used in proportions of between 0.1 and 10% by weight, the inorganic or organic particles representing 0.075 to 70% by weight, the remainder of the mixture consisting of water or a water/solvent mixture.

11. Process according to claim 1, wherein the indoline is selected from the group consisting of: 5,6-dihydroxyindoline, 6-hydroxy indoline, 5,6-methylenedioxyindoline, 7-methoxy-6-hydroxy indoline, 6,7-dihydroxyindoline, 5-hydroxy-4-methoxy indoline, 4,5-dihydroxyindoline, 5-methoxy-6-hydroxy indoline, 4-hydroxy-5-methoxyindoline, 5-hydroxy-6-methoxy indoline, 4,7-dihydroxyindoline, 6-aminoindoline, N-ethyl-

4-hydroxyindoline, 1-ethyl-6-amino-indoline, 5,6-diaminoindoline, 1-methyl-6-aminoindoline, 2-methyl-6-aminoindoline, 3-methyl-6-aminoindoline, 2-methyl-5,6-diaminoindoline, 5-chloro-7-aminoindoline, 3-methyl-5,7-diaminoindoline, 5,7-diaminoindoline, 2-methyl-5,7-diaminoindoline, 7-aminoindoline, 2-methyl-7-amino indoline, 4-aminoindoline, 4-amino-6-chloroindoline, 4-amino-6-iodoindoline, 4-amino-5-bromoindoline, 4-amino-5-hydroyindoline, 4-amino-7-hydroxyindoline, 4-amino-5-methoxyindoline, 4-amino-7-methoxyindoline, 5-amino indoline, 2,3-dimethyl-5-aminoindoline, 1-methyl-5-aminoindoline, 2-methyl-5-aminoindoline, 5-[N-(1-methyl hexyl)amino]indoline, 5,6-dimethoxyindoline and 5,6-dihydroxy-2-carboxyindoline.

12. Process according to claim 1 wherein the inorganic or organic particles are selected from the group consisting of lamellar or non-lamellar inorganic particles and lamellar or non-lamellar organic particles, the inorganic or organic particles being colored or uncolored, these particles having an average particle size of between 0.01 and 200 microns.

13. Process according to claim 1 wherein the non-lamellar inorganic particles are inert inorganic particles having a particle size of less than 20 microns.

14. Process according to claim 13, wherein the non-lamellar inorganic particles are calcium carbonate, silica or titanium oxide particles.

15. Process according to claim 1, wherein the particles are inorganic or organic lamellar particles which take the form of lamellae, the ratio of the largest dimension to the thickness being between 2 and 100 and the largest dimension being less than 50 microns.

16. Process according to claim 15, wherein the lamellar particles are selected from the group consisting of L-lauroyllysine, microparticles of ceramic which are optionally coated with zirconium powder, lamellar titanium dioxide, lamellar talc, boron nitride, lamellar mica, bismuth oxychloride and transparent red iron oxide.

17. Process according to claim 1, wherein the particles are colored inorganic particles consisting of metal salts which are insoluble in the cosmetic medium having a particle size of between 0.01 and 150 microns.

18. Process according to claim 17 wherein the colored inorganic particles are selected from the group consisting of iron oxides, ultramarine blue, chromium oxides, manganese violet and Prussian blue.

19. Process according to claim 1, wherein the particle is a nacreous or interference pigment having a particle size of between 10 and 150 microns.

20. Process according to claim 1, wherein the organic particles are non-lamellar organic particles having a particle size of less than 100 microns and which contain the indoline-based product at the surface or in the polymer network or at the surface and in the polymer network.

21. Process according to claim 20, wherein the particles of polymers are selected from the group comprising of:

(a) polymers derived from keratin;

(b) silk fibroins;

(c) chitin or chitosan;

(d) microcrystalline cellulose;

(e) synthetic polymers selected from the group consisting of:

(i) polyethylene, polypropylene, polystyrene and poly-(methyl methacrylate) which is optionally crosslinked;

(ii) crosslinked poly-β-alanine;

(iii) styrene/divinylbenzene, methyl methacrylate/ethylene glycol dimethylacrylate and vinyl stearate/divinylbenzene crosslinked polymers;

(iv) hollow microspheres of the copolymer of vinylidene chloride and acrylonitrile;

(v) porous microspheres of polyamide 12, polyamide or copolyamide 6/12; and

(vi) silicone powder consisting of gums, resins and organosiloxane elastomers.

* * * * *

US006323367B1

(12) **United States Patent**
Choudary et al.

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(54) **PROCESS FOR THE PREPARATION OF AMINE OXIDES**

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(58) **Field of Search** 564/297, 298; 546/141, 188, 189; 544/106, 107

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(57) **ABSTRACT**

A process for the preparation of high quality amine oxides by reacting a tertiary or secondary amine with hydrogen peroxide as an oxidant in the presence of a recyclable heterogeneous layered double hydroxide exchanged with one of the anions of transition metal oxides as a catalyst in an organic solvent at a temperature ranging between 10–25° C. for a period of 1–6 hours under continuous stirring and separating the product by simple filtration and subsequently evaporation of solvents by known methods.

8 Claims, No Drawings

PROCESS FOR THE PREPARATION OF AMINE OXIDES

FIELD OF THE INVENTION

The present invention relates to a process for the preparation of high quality amine oxides from secondary and tertiary aliphatic amines. More particularly, the present invention relates to an improved process for the preparation of amine oxides from secondary and tertiary aliphatic amines useful in the preparation of hair conditioners and shampoos, toothpaste, laundry detergent powder, fabric softeners, toilet soap bars and cosmetics, surfactants as well as in other applications as synthetic intermediates and excellent spin trapping reagents.

BACKGROUND OF THE INVENTION

N-oxides hold a key position in the chemistry of heterocycles as well as in biomedical area. Tertiary amine oxides are widely used in treatment of fabrics and preparation of hair conditioners and shampoos, toothpaste, laundry detergent powder, fabric softeners, toilet soap bars and cosmetics as well as in other applications. They were also used as stoichiometric oxidants in metal catalysed hydroxylation and epoxidation reactions of olefins. On the other hand, oxides derived from secondary amines, called nitrones are highly valuable synthetic intermediates and excellent spin trapping reagents. In particular nitrones are excellent 1,3 dipoles and have been utilized for the synthesis of various nitrogen containing biologically active compounds e.g. alkaloids and lactams.

Conventionally tertiary amine oxides are prepared by oxidation of respective tertiary amines with strong oxidising agent like aqueous hydrogen peroxide in a solvent such as water, lower alcohol, acetone or acetic acid. A dilute or preferably concentrated (30–90% by weight) hydrogen peroxide solution is added in stoichiometric or greater amount to an aqueous solution containing the tertiary amine to obtain amine oxide, (U.S. Pat. No. 3,215,741). The drawback associated with this process is the formation of a gel resembling a thick paste long before completion of the reaction, which retards further reaction. The yields are only 30–40% by weight of amine oxide. Several other methods such as incorporation of catalyst and/chelating agent have been developed in order to increase the quality and yields of the product.

In case of secondary amines, the classical methods involve the condensation of N-monosubstituted hydroxylamines with carbonyl compounds or the direct oxidation of N,N-disubstituted hydroxylamines. Subsequently, direct oxidation of secondary amines using several oxidising systems such as $R_2C(\mu\text{-}O_2)$, $Na_2WO_4—H_2O_2$, SeO_2, TPAP-NMO and UHP-M (M=Mo, W), $MTO\text{-}H_2O_2$ have been developed to prepare nitrones under homogenous conditions. The drawback in all the above cases is the difficulty in recovering the homogeneous catalyst/reagents from the reaction mixture.

Reference is made to U.S. Pat. No. 3,283,007 wherein the oxidation of tertiary amines using diethelene triamine penta/tetra acetic acid as chelating agent and sometimes contaminated with heavy metals is recommended to improve the yield. The hydrogen peroxide solution employed has concentration of at least 30–75% by weight. The disadvantages of this process are high reaction temperatures ranging between 40–100° C., longer reaction periods, and lower yields of amine oxides.

Reference is made to U.S. Pat. No. 3,424,780, wherein high yields of tertiary amine oxides are achieved by carrying

the oxidation of tertiary amine with 30–70% by weight of aqueous hydrogen peroxide using 0.01 to 2% weight of carbondioxide, in presence of a chelating agent, tetra acetylene diamine, a salt thereof, polyphosphates, stannates, a hydroxy carboxylic acid salts or the salt of poly carboxylic acid. The reaction is carried out at a temperature ranging from 40 to 80° C. The disadvantages of this process are high reaction temperature, longer reaction periods and that the amine oxide formed is intensively coloured when carbon dioxide atmosphere is used to speed up the reaction and this method necessitates injecting a gas which requires handling facilities. Another disadvantage is that the presence of more than 30% by weight of hydrogen peroxide is not environmentally friendly.

Reference is made to another U.S. Pat. No. 4,889,954 wherein the tertiary amines are reacted in high yields to give the corresponding amine oxides with a low content of nitrosamine, the oxidation of tertiary amine being carried out in the presence of a dialkyl carboxylic acid ester as catalyst and if appropriate, ascorbic acid as a co-catalyst using 45–70% by weight of hydrogen peroxide. The drawbacks in the above process are the requirement of frequent addition of water to avoid gel formation, high reaction temperatures, longer reaction periods and difficulty in separation of the catalyst from the reaction mixture.

Reference is made to another U.S. Pat. No. 4,565,891 wherein octacyano molybdate or iron salts are used as catalysts and molecular oxygen for oxidation of tertiary amines at high pressures and temperatures. The main drawback of this process is the need of very high temperature of 90–130° C. and very low yields of amine oxide reporting 11–52% of conversion.

Reference is made to a U.S. Pat. No. 5,130,488 wherein the solid amine oxide can be prepared by reacting a tertiary amine with hydrogen peroxide using carbon dioxide in presence of acetate and cooling to precipitate the product. This process is superior to previously known methods of preparing amine oxides. However, its use can sometimes lead to cleavage of the solvents, plating on the walls of the vessel used for the precipitation, contamination of the product with residual peroxide, and or discoloration of the product.

Reference is made to a publication by Walter W. Zajac et al., J. Org. Chem.; 53, 5856, 1988 wherein the oxidation of secondary and tertiary amines using 2-sulfonyloxyxaziridines (Davis Reagents) were reported. The drawback of the above process is that the reagent is used in stoichiometric amounts.

Reference is made to a publication by Shun-Ichi Murahashi et al., J. Org. Chem.; 55, 1736, 1990 wherein sodium tungstate is used as catalyst for the oxidation of secondary amines. The drawback is the difficulty in recovery of the catalyst from homogeneous conditions.

Reference is also made to publication by Murraay et al., J. Org. Chem., 61, 8099, 1996 wherein methyltrioxorhenium was used as a catalyst in oxidation of secondary amines. The drawback is the difficulty in recovery of the catalyst.

OBJECTS OF THE INVENTION

The main object of the present invention is to provide an eco-friendly and simple process for N-oxidation of secondary and tertiary amines using layered double hydroxides exchanged with anions of transition metal oxides as a catalyst, which is cheaper, non-corrosive and recyclable catalyst utilising only lower percentage of hydrogen peroxide at room temperatures to give high yields of product.

Another object of the present invention is to provide an improved process for the preparation of tertiary amine oxides and secondary amine oxides (nitrones), widely used in detergents, shampoos, fabric softers and biomedical area.

Another object of the present invention is the use of non-corrosive and low cost heterogeneous catalyst i.e. layered double hydroxides exchanged with tungstate, molybdate, vanadate and their polyanions.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides an improved process for the preparation of amine oxides which comprises reacting tertiary and secondary amines with hydrogen peroxide as an oxidant in presence of a recyclable heterogeneous catalyst comprising layered double hydroxides exchanged with anion of transition metal oxides selected from tungstate, molybdate, vanadate, and their polyanions, in an organic solvent at a temperature in the range between 10–25° C. for a period of 1–6 hours under continuous stirring and separating the product by simple filtration and subsequently evaporation of solvents by known methods.

In an embodiment of the present invention, the heterogeneous catalyst used is the layered double hydroxides exchanged with transition metal oxides selected from a group consisting of tungstate, molybdate, vanadate and their polyanions i.e. polyoxometalates having formula I: $[M^{II}_{(1-x)}M^{III}_{x}(OH)_2][M^{n-}]_{x/2}.zH_2O$, which is derived from LDH having formula II $[M^{II}_{(1-x)}M^{III}_{x}(OH)_2][A^{n-}]_{x/2}.zH_2O$ where M is a transition metal oxides selected from the group consisting of W, Mo, V and A^{n-} is interstitial anion, selected from nitrate and chloride and M^{II} is a divalent cation selected from the group consisting of Mg^{2+}, Mn^{2+}, Fe^{2+}, V^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Ca^{2+} and M^{III} is a trivalent ion selected from the group consisting of Al^{3+}, Cr^{3+}, V^{3+}, Mn^{3+}, Fe^{3+} and Co^{3+}, x is the mole fraction having integral value ranging from 0.2 to 0.33, and z is the number of water molecules and ranges from 1 to 4.

In another embodiment of the present invention, the tertiary amines used have the general formula $R^1R^2NR^3$ wherein R^1, R^2 and R^3, which may be the same or different, and are the straight-chain or branched-chain groups selected from alkyl, alkenyl and aralkyls having C_1–C_{24} carbons selected from N,N- dimethyl decyl amine, N,N-dimethyl dodecyl amine, N,N-dimethylbenzylamine, triethylamine, tributylamine and cyclic amines selected from imidazolines pyridines, N-substituted piperazines, N-substituted piperadines or N-substituted morpholines, e.g., N-methylmorpholine.

In another embodiment of the present invention, the secondary amines used have the general formula R^1R^2NH wherein R^1 and R^2 may be the same or different and are the straight-chain or branched-chain groups selected from alkyl, alkenyl and aralkyls having C_1–C_{24} carbons, selected from dibutyl amine, dibenzyl amine, N-benzyl phenethylamine, N-phenyl benzylamine and cyclic amines selected from piperidine, 1,2,3,4, tetrahydro isoquinoline.

In another embodiment of the present invention aqueous hydrogen peroxide is added slowly in a controlled manner for a period ranging between 0–120 min.

In yet another embodiment of the present invention, the catalyst introduced in the system is 6–12% by weight of anion of transition metal oxides selected from tungstate, molybdate, vanadate and their polyanions as polyoxometalates.

In still another embodiment of the present invention, water miscible organic solvent used is selected from group consisting of methonol, ethanol, isopropanol, 1-propanol, 1-butanol, 2-butanol and isobutyl alcohol.

In still another embodiment of the present invention, the amount of hydrogen peroxide used is 2 to 6 moles per mole of amine.

DETAILED DESCRIPTION OF THE INVENTION

The catalyst of the invention comprises a recyclable heterogeneous catalyst, i.e. layered double hydroxides exchanged with tungstate, molybdate, vanadate and their polyanions i.e. polyoxometalates that catalyses oxidation of secondary and tertiary amines. The advantages such as low cost of the catalyst, reusability for several times and its ability to oxidise the amines at 10–25° C., below or at room temperature in a shorter period make the present invention as a promising candidate for a clean and efficient industrial route to amine oxide preparation.

The novelty of the invention lies in the use of heterogeneous catalyst for the first time for the N-oxidation of secondary and tertiary amines. The anion of transition metal oxides intercalated in the layered double hydroxide effectively catalyses the oxidation of amines to amine oxides. The catalyst was removed by simple filtration and the solid catalyst obtained thus is recycled for several times without any addition of fresh catalyst. The consistent activity for several cycles, mild reaction conditions, shorter reaction times makes the process economical and possible for commercial realisation.

According to the invention, amine oxides are prepared by reacting tertiary and secondary amines with hydrogen peroxide as an oxidant in presence of a recyclable heterogeneous catalyst comprising layered double hydroxides exchanged with anion of transition metal oxides selected from tungstate, molybdate, vanadate, and their polyanions, in an organic solvent at a temperature in the range between 10–25° C. for a period of 1–6 hours under continuous stirring. The product is separated by simple filtration and the solvents evaporated by known methods.

The heterogeneous catalyst used are layered double hydroxides exchanged with transition metal oxides selected from a group consisting of tungstate, molybdate, vanadate and their polyanions i.e. polyoxometalates having formula I: $[M^{II}_{(1-x)}M^{III}_{x}(OH)_2][M^{n-}]_{x/2}.zH_2O$, which is derived from LDH having formula II $[M^{II}_{(1-x)}M^{III}_{x}(OH)_2][A^{n-}]_{x/2}.zH_2O$ where M is a transition metal oxides selected from the group consisting of W, Mo, V and A^{n-} is interstitial anion, selected from nitrate and chloride and M^{II} is a divalent cation selected from the group consisting of Mg^{2+}, Mn^{2+}, Fe^{2+}, V^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Ca^{2+} and M^{III} is a trivalent ion selected from the group consisting of Al^{3+}, Cr^{3+}, V^{3+}, Mn^{3+}, Fe^{3+} and Co^{3+}, x is the mole fraction having integral value ranging from 0.2 to 0.33, and z is the number of water molecules and ranges from 1 to 4.

The tertiary amines used have the general formula $R^1R^2NR^3$ wherein R^1, R^2 and R^3, which may be the same or different, and are the straight-chain or branched-chain groups selected from alkyl, alkenyl and aralkyls having C_1–C_{24} carbons selected from N,N-dimethyl decyl amine, N,N-dimethyl dodecyl amine, N,N-dimethylbenzylamine, triethylamine, tributylamine and cyclic amines selected from imidazolines pyrididines, N-substituted piperazines, N-substituted piperadines or N-substituted morpholines, e.g., N-methylmorpholine.

The secondary amines used have the general formula R^1R^2NH wherein R^1 and R^2 may be the same or different

and are the straight-chain or branched-chain groups selected from alkyl, alkenyl and aralkyls having C_1–C_{24} carbons, selected from dibutyl amine, dibenzyl amine, N-benzyl phenethylamine, N-phenyl benzylamine and cyclic amines selected from piperidine, 1,2,3,4, tetrahydro isoquinoline.

Aqueous hydrogen peroxide is added slowly in a controlled manner for a period ranging between 0–120 min. The catalyst introduced in the system is generally 6–12% by weight of anion of transition metal oxides selected from tungstate, molybdate, vanadate and their polyanions as polyoxometalates. The water miscible organic solvents are selected from group consisting of methonol, ethanol, isopropanol, 1-propanol, 1-butanol, 2-butanol and isobutyl alcohol. The amount of hydrogen peroxide used may be in the range of 2 to 6 moles per mole of amine.

The catalytic cycle in the oxidation of amines to amine oxides involves the easy formation of peroxotungstate, $HOOWO_3^-$/$HOOWO_6^-$ on interaction of tungstate with hydrogen peroxide. These peroxy species will act as an active species for the oxidation of secondary/tertiary amines as described by Murahashi et. al., for the Na_2WO_4 catalysed oxidation of secondary amines by hydrogen peroxide. The secondary amine undergoes nucleophilic reaction with peroxotungstate species to give hydroxylamine. Further oxidation of hydroxylamine followed by dehydration gives nitrone. In case of tertiary amines, the oxygen transfer occurs from peroxotungstate species to tertiary amine in a single step to form tertiary amine oxide. The species $HOWO_3^-$/$HOWO_6^-$ thus formed is readily oxidized with another molecule of H_2O_2 to give peroxo tungstate $HOOWO_3^-$/$HOOWO_6^-$, thus completing the catalytic cycle.

The following examples are given by way of illustration of the present invention and therefore should not be construed to limit the scope of the present invention.

EXAMPLE 1

Preparation of the Various Catalysts

1. Preparation of Mg—Al Hydrotalcite (LDH) Chloride:

Mg—Al—Cl hydrotalcite (3:1) is prepared as follows: About 200 ml of decarbonated and deionised water was taken into a 1 liter four necked round bottomed flask and stirred at 25° C. with a magnetic stirrer under a nitrogen flow. The mixture (Al^{3+}=0.05 mol/l), (Mg^{2+}=0.15 mol/l) of decarbonated solution of $AlCl_3.9H_2O$ (12.07 g), $MgCl_2.6H_2O$ (30.49 g) (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland) and aqueous solution of sodium hydroxide (166 g, 0.2 mol/l) were added continuously drop-wise from a burette, the pH of the reaction mixture being kept at 10.00–10.2 during the reaction. The precipitate obtained was filtered, washed with deionised and decarbonated water and dried at 70° C. for 15 h.

a)) Preparation of (Mg—Al Hydrotalcite (LDH) Tungstate (Catalyst A):

To reach anion exchange of degree of 12%, 1 g of Mg—Al—Cl hydrotalcite was stirred in 100 ml of aqueous solution of 1.87 mM (0.616 g) sodium tungstate (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland), at 293K for 24 h. The solid catalyst was filtered, washed with deionised and decarbonated water and lyophilized to dryness.

b) Preparation of Mg—Al Hydrotalcite (LDH) Molybdate (Catalyst C):

To reach anion exchange of degree of 12%. 1 g of Mg—Al—Cl hydrotalcite was stirred in 100 ml of aqueous solution of 1.87 mM (0.452 g) sodium molybdate (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland), at 293K for 24 h. The solid catalyst was filtered, washed with deionised and decarbonated water and lyophilized to dryness.

c) Preparation of Mg—Al Hydrotalcite (LDH) Vanadate (Catalyst C):

To reach anion exchange of degree of 12%, 1 g of Mg—Al—Cl hydrotalcite is stirred in 100 ml of aqueous solution of 1.87 mM (0.456 g) sodium vanadate (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland), at 293K for 24 h. The solid catalyst was filtered, washed with deionised and decarbonated water and lyophilized to dryness.

2. Preparation of Mg—Al Hydrotalcite (LDH) Nitrate:

Magnesium nitrate hexahydrate (30.8 g, 0.12 mol) and aluminium nitrate nonahydrate (15.0 g, 0.04 mol) were dissolved in 100 ml of deionised and decarbonated water. The pH of the solution was adjusted to 10 by adding 2M NaOH. The resulting suspension was stirred for 2 h at room temperature. The precipitate hydrotalcite was collected by filtration under N_2 atmosphere and dried overnight at 80° C.

a) Preparation of Mg—Al hydrotalcite (LDH) tungstate (Catalyst D):

To reach anion exchange of degree of 12%, 1 g of Mg—Al—NO_3 hydrotalcite was stirred in 100 ml of aqueous 1.87 mM (0.616 g) sodium tungstate (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland), at 293K for 24 h. The solid catalyst was filtered, washed with of deionised and decarbonated water and lyophilized to dryness.

3. Preparation of Mg—Al hydrotalcite (LDH) carbonate:

Mg—Al—CO_3 hydrotalcite (3:1) is prepared as follows: An aqueous solution (0.280 l) containing $Mg(NO_3)_2.6H_2O$ (0.2808 mol) and $Al(NO_3)_3.9H_2O$ (0.093 mol) from M/s. Fluka, a Sigma Aldrich Company, Switzerland) was added slowly to a second solution (0.280 l) containing NaOH (0.6562 mol) and Na_2CO_3 (0.3368 mol) in a 1.0 l round bottomed flask under vigorous stirring. The addition took nearly 3 h. Then the slurry was heated to 338 K for 16 h. The precipitate formed was filtered off and washed with hot distilled water until the pH of the filtrate was 7. The precipitate was dried in an oven at 353K for 15 h.

a) Preparation of Mg—Al hydrotalcite (LDH) tungstate (Catalyst E):

To reach anion exchange of degree of 12%, 1 g of Mg—Al— CO_3 calcined (at 723 K for 6 h in a flow of air) hydrotalcite was stirred in 100 ml of aqueous solution of 1.87 mM (0.616 g) sodium tungstate (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland), at 293K for 24 h. The solid catalyst was filtered, washed with deionised and decarbonated water and lyophilized to dryness.

4. Preparation of Ni—Al Hydrotalcite (LDH) Chloride:

Ni—Al hydrotalcite chloride (3:1) was prepared as follows: About 200 ml of decarbonated and deionised water was taken into a 1 liter four necked round bottomed flask and stirred at 25° C. with a magnetic stirrer under nitrogen flow. A mixture (Al^{3+}=0.05 mol/l), (Ni^{2+}=0.15mol/l) of decarbonated solution of $AlCl_3.9H_2O$ (12.07 g), $NiCl_2.6H_2O$ (35.65 g) (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland) and aqueous solution of sodium hydroxide (16 g, 0.2 mol/l) were added continuously drop-wise from a burette, the pH of the reaction mixture being kept at 10.00–10.2 during the reaction. The precipitate obtained was filtered, washed with deionised and decarbonated water and dried at 70° C. for 15 h.

a) Preparation of Ni—Al hydrotalcite (LDH) tungstate (Catalyst F):

To reach anion exchange of degree of 12%, 1 g of Ni—Al hydrotalcite chloride was stirred in 100 ml of aqueous 1.87

mM (0.616 g) sodium tungstate (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland), at room temperature for 24 h. The solid catalyst was filtered, washed with deionised and decarbonated water and lyophilized to dryness.

5. Preparation of Ni—Al hydrotalcite (LDH) Nitrate:

Nickel nitrate hexahydrate (34.8 g, 0.12 mol) and aluminium nitrate nonahydrate (15.0 g, 0.04 mol) were dissolved in 100 ml of deionised and decarbonated water. The pH of the solution was adjusted to 10 by adding 2M NaOH. The resulting suspension was stirred for 2 h at room temperature. The precipitate hydrotalcite was collected by filtration under N_2 atmosphere and dried overnight at 80° C.

a) Preparation of Ni—Al hydrotalcite (LDH) tungstate (Catalyst):

To reach anion exchange of degree of 12%, 1 g of Ni—NO—Al—$_3$ hydrotalcite was stirred in 100 ml of aqueous 1.87 mM (0.616 g) sodium tungstate (obtained from M/s. Fluka, a Sigma Aldrich Company, Switzerland), at 293K for 24 h. The solid catalyst was filtered, washed with of deionised and decarbonated water and lyophilized to dryness.

EXAMPLE 2

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The four-necked flask was charged with 0.22 ml (2 mmol) of N-methylmorpholine, 200 mg of catalyst A and 50 ml of methanol. To the mixture was added dropwise 6.6 ml (6 mmol) of a 30% by weight of aqueous solution of hydrogen peroxide for period of 2.0 hours in 2 to 3 portions at 25° C. under continuous stirring. Continued the reaction for another 0.5 hour. After the completion of the reaction (followed by TLC), the catalyst was filtered off and washed with methanol. To the filtrate a small amount of manganese dioxide was added to decompose the unreacted hydrogen peroxide. The treated reaction mixture was filtered to remove the solid MnO_2 and concentrated under reduced pressure to obtain the product. The product thus obtained was purified by column chromatography to afford the corresponding amine oxide. N-methylmorpholine N-oxide of 98% yield was obtained. This product is commercially available from Fluka, Aldrich, Lancaster and Merck companies.

EXAMPLE 3

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide: Recycle-I

The oxidation reaction of N-methylmorpholine by using catalyst A which had been used in example 2 was performed in an identical procedure as in example 2, without further addition of fresh catalyst. N-methylmorpholine N-oxide of 98% yield was obtained.

EXAMPLE 4

Oxidation, of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide: Recycle-II

The oxidation reaction of N-methylmorpholine by using catalyst A which had been used in example 3 was performed in an identical procedure as in Example 2, without further addition of fresh catalyst. N-methylmorpholine N-oxide of 96% yield was obtained.

EXAMPLE 5

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide. Recycle-III

The oxidation reaction of N-methylmorpholine by using catalyst A which had been used in example 4 was performed in an identical procedure as in example 2, without further addition of fresh catalyst. N-methylmorpholine N-oxide of 97% yield was obtained.

EXAMPLE 6

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide: Recycle-III

The oxidation reaction of N-methylmorpholine by using catalyst A which had been used in example 5 was performed in an identical procedure as in example 2, without further addition of fresh catalyst. N-methylmorpholine N-oxide of 96% yield was obtained.

EXAMPLE 7

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide: Recycle-IV

The oxidation reaction of N-methylmorpholine by using catalyst A which had been used in reaction 6 in an identical procedure as in example 2, without further addition of fresh catalyst. N-methylmorpholine N-oxide of 96% yield was obtained.

EXAMPLE 8

Oxidation of Tributyl Amine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of triethyl amine by using catalyst A was performed in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. Triethyl amine N-oxide of 98% yield was obtained.

EXAMPLE 9

Oxidation of Tributyl Amine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of tributyl amine was performed by using catalyst A in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. Tributyl amine N-Oxide of 95% yield was obtained.

EXAMPLE 10

Oxidation of N,N-dibutyl benzylamine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N,N-dibutyl benzylamine was performed by using catalyst A in an identical procedure as in

example 3. The time taken for the completion of reaction was 3 hours. N,N-dibutyl benzyl amine N-oxide of 96% yield was obtained.

EXAMPLE 11

Oxidation of N-benzyl Piperidine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N-benzyl piperidine was performed by using catalyst A, in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. N-benzyl piperidine N-oxide of 98% yield was obtained.

EXAMPLE 12

Oxidation of N,N-dimethyldecylamine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N,N-dimethyldecylamine was performed by using catalyst A in an identical procedure as in example 2. N,N-dimethyldecylamine N-oxide of 98% yield was obtained. This product is commercially available from Lonza Inc., With trade name Barlox 10S (Specification: 30 weight percent decyldimethyl tertiary amine oxide).

EXAMPLE 13

Oxidation of N,N-dimethyloctylamine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N,N-dimethyloctylamine was performed by using catalyst A in an identical procedure as in example 3. N,N-dimethyloctylamine N-oxide of 98% yield was obtained.

EXAMPLE 14

Oxidation of N,N-dimethyl benzylamine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N,N-dimethyl benzylamine was performed by using catalyst A in an identical procedure as in Example 2. The time taken for the completion of reaction was 3 hours. N,N-dimethyl benzylamine amine N-oxide of 95% yield was obtained.

EXAMPLE 15

Oxidation of N,N-dimethylcyclohexylamine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N,N-dimethylcyclohexylamine by using catalyst A was performed in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. N,N-dimethylcyclohexylamine N-oxide of 97% yield was obtained.

EXAMPLE 16

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N-methylmorpholine was performed using catalyst B in an identical procedure as in Example 2. N-methylmorpholine N-oxide of 90% yield was obtained.

EXAMPLE 17

Oxidation of N-methyl morpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N-methylmorpholine by using catalyst (was performed in an identical procedure as in example 2. N-methylmorpholine N-oxide of 40% yield was obtained.

EXAMPLE 18

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N-methylmorpholine by using catalyst D was performed in an identical procedure as in example 2. N-methylmorpholine N-oxide of 96% yield was obtained.

EXAMPLE 19

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N-methylmorpholine by using catalyst E was performed in an identical procedure as in example 2. N-methylmorpholine N-oxide of 95% yield was obtained.

EXAMPLE 20

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Ni/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N-methylmorpholine was performed using catalyst F in an identical procedure as in example 2. N-methylmorpholine N-oxide of 98% yield was obtained

EXAMPLE 21

Oxidation of N-methylmorpholine Catalysed by Tungstate Exchanged with Ni/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N-methylmorpholine was performed using catalyst G in an identical procedure as in example 2. N-methylmorpholine N-oxide of 96% yield was obtained.

EXAMPLE 22

Oxidation of Dibutyl Amine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of dibutyl amine was performed by using catalyst D in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. N-butylidene-butylamine N-oxide of 96% yield was obtained.

EXAMPLE 23

Oxidation of Dibutyl Amine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of dibutyl amine was performed by using catalyst E in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. N-butylidene-butylamine N-oxide of 95% yield was obtained.

EXAMPLE 24

Oxidation of Dibutyl Catalysed by Tungstate Exchanged with Ni/Al (3:1) L Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of dibutyl amine was performed by using catalyst A in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. N-butylidene-butylamine N-oxide of 96% yield was obtained.

EXAMPLE 25

Oxidation of Dibutyl Amine Catalysed by Tungstate Exchanged with Ni/Al (3:3) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of dibutyl amine was performed by using catalyst G in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. N-butylidene-butylamine N-oxide of 95% yield was obtained.

EXAMPLE 26

Oxidation of Dibutyl Amine Catalysed by Tungstate Exchanged with Ni/Al (3:3) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of dibutyl amine was performed by using catalyst A in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. N-butylidene-butylamine N-oxide of 97% yield was obtained.

EXAMPLE 27

Oxidation of Dibutyl Amine Catalysed by Tungstate Exchanged with Ni/Al (3:3) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of dibenzyl amine was performed by using of catalyst A in an identical procedure as in example 2. The time taken for the completion of reaction was 5 hours. N-benzylidenebenzylamine N-oxide of 60% yield was obtained.

EXAMPLE 28

Oxidation of N-benzyl Phenethylamine Catalysed by Tungstate Exchanged with Mg/Al (3:3) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of N-benzyl phenethylamine was performed by using catalyst A in an identical procedure as in example 2. The time taken for the completion of reaction was 6 hours. N-(1-methyl benzylidine) phenylamine N-oxide of 90% yield was obtained.

EXAMPLE 29

Oxidation of N-phenyl Benzylamine Amine Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The reaction oxidation reaction of N-phenyl benzylamine was performed by using catalyst A in an identical procedure as in example 2. The time taken for the completion of reaction was 4 hours. N-bezylidine phenylamine N-oxide of 93% yield was obtained.

EXAMPLE 30

Oxidation of Piperidine Catalysed by Tungstate Exchanged with Ni/Al (3:1) Layered Double Hydroxides Using Aqueous Hydrogen Peroxide

The oxidation reaction of piperidine by using catalyst A was performed in an identical procedure as in example 2. The time taken for completion of reaction was 3 hours. 2,3,4,5 tetrahydro pyridine N-oxide of 92% yield was obtained.

EXAMPLE 31

Oxidation 1,2,3,4-Tetrahydroisoquinoline Catalysed by Tungstate Exchanged with Mg/Al (3:1) Layered Double Hydroxides Using Aqueous hydrogen Peroxide

The oxidation reaction of 1,2,3,4-tetrahydroisoquinoline performed by using catalyst A in an identical procedure as in example 2. The time taken for completion of reaction was 5 hours. 3,4 dihydroisoquinoline N-oxide of 93% yield was obtained.

EXAMPLE 32

Oxidation of Diisopropyl Amine Catalysed Tungstate Exchanged with Mg/Al (3:1) Layered double Hydroxides Using Aqueous Hydrogen Peroxide

The reaction oxidation reaction of diisopropyl amine was performed by using catalyst A in an identical procedure as in example 2. The time taken for the completion of reaction was 3 hours. N-(1-methylethylidine)-1-methylethylamine N-oxide of 92% yield was obtained.

The main advantages of the present invention are:

1. The present process is eco-friendly and very simple.
2. The catalyst is cheap, non-corrosive, recyclable for several times and heterogeneous in nature.
3. The reaction conditions are very mild, being the reaction temperature ranges between 10–25°C.
4. The hydrogen peroxide used is 30% by weight, which is more environmentally friendly.
5. The process is economical.
6. The process is accomplished in a short time to afford high productivity.
7. The amount of effluents formed in this process is minimized because the catalyst and solvent are recovered/recycled and reused.
8. The process provides high quality of the product without resulting in gel formation, ring the course of reaction.

TABLE 1

Reusability of the catalyst in the oxidation of N-methylmorpholine catalysed by tungstate exchanged with Mg/Al (3:1) layered double hydroxides (catalyst A) using aqueous hydrogen peroxide[a]

Ex. No	Tertiary amine	Cycle	Time (h)	Amine oxide	Yield[b]
2	N-methylmorpholine	1	2.5	N-methylmorpholine N-oxide	98
3	N-methylmorpholine	2	2.5	N-methylmorpholine N-oxide	98
4	N-methylmorpholine	3	2.5	N-methylmorpholine N-oxide	96
5	N-methylmorpholine	4	2.5	N-methylmorpholine N-oxide	97
6	N-methylmorpholine	5	2.5	N-methylmorpholine N-oxide	96
7	N-methylmorpholine	6	2.5	N-methylmorpholine N-oxide	96

[a]Reaction conditions as exemplified in example 2
[b]Isolated yields

TABLE 2

Oxidation of tertiary amines catalysed by anion of transition metal oxides exchanged layered double hydroxides using aqueous hydrogen peroxide[a]

Ex. No	Tertiary amine	Catalyst	Amine oxide	Time (h)	Yield[b]
8	Triethyl amine	A	Triethyl amine N-oxide	3.0	98
9	Tributyl amine	A	Tributyl amine N-oxide	3.0	95
10	N,N-dibutyl benzyl amine	A	N,N-dibutyl benzyl amine N-oxide	3.0	96
11	N-benzyl piperidine	A	N-benzyl piperidine N-oxide	3.0	98
12	N,N-dimethyl decyl amine	A	N,N-dimethyl decyl amine N-oxide	2.5	98
13	N,N-dimethyl octyl amine	A	N,N-dimethyl octyl amine N-oxide	2.5	98
14	N,N-dimethyl benzyl amine	A	N,N-dimethyl benzyl amine N-oxide	3.0	95
15	N,N-dimethyl cyclohexylamine	A	N,N-dimethyl cyclohexyl amine N-oxide	3.0	97
16	N-methyl morpholine	B	N-methylmorpholine N-oxide	2.5	90
17	N-methyl morpholine	C	N-methylmorpholine N-oxide	2.5	40
18	N-methyl morpholine	D	N-methylmorpholine N-oxide	2.5	96
19	N-methyl morpholine	E	N-methylmorpholine N-oxide	2.5	95
20	N-methyl morpholine	F	N-methylmorpholine N-oxide	2.5	98
21	N-methyl morpholine	G	N-methylmorpholine N-oxide	2.5	96

[a]Reaction conditions as exemplified in example 2
[b]Isolated yields

TABLE 3

Oxidation of secondary amines catalysed by anion of transition metal oxides exchanged layered double hydroxides using aqueous hydrogen peroxide[a]

Ex. No	Secondary amine	Catalyst	Amine oxide (nitrone)	Time (h)	Yield[b]
22	Dibutyl amine	D	N-butylidene-butylamine N-oxide	3	96
23	Dibutyl amine	E	N-butylidene-butylamine N-oxide	3	95
24	Dibutyl amine	F	N-butylidene-butylamine N-oxide	3	96

TABLE 3-continued

Oxidation of secondary amines catalysed by anion of transition metal oxides exchanged layered double hydroxides using aqueous hydrogen peroxide[a]

Ex. No	Secondary amine	Catalyst	Amine oxide (nitrone)	Time (h)	Yield[b]
25	Dibutyl amine	G	N-butylidene-butylamine N-oxide	3	95
26	Dibutyl amine	A	N-butylidene-butylamine N-oxide	3	97
27	Dibenzyl amine	A	N-benzylidene benzylamine N-oxide	5	60
28	N-benzyl phenethyl amine	A	N-(1-methylbenzylidene) benzylamine N-oxide	6	90
29	N-Phenyl benzyl amine	A	N-benzylidene phenylamine N-oxide	4	93
30	Piperidine	A	2,3,4,5 Tetrahydro pyridine N-oxide	3	92
31	1,2,3,4 Tetrahydro isoquinoline	A	3,4, Dihydroisoquinoline N-oxide	5	93
32	Diisopropyl amine	A	N-(1-ethylethylidene)1-methylethyl amine N-oxide	3	92

[a]Reaction conditions as exemplified in example 2
[b]Isolated yields

We claim:

1. A process for the preparation of high quality amine oxides which comprises reacting a tertiary or secondary amine with hydrogen peroxide as an oxidant in presence of a recyclable heterogeneous layered double hydroxide exchanged with one of the anions of transition metal oxides as a catalyst in an organic solvent at a temperature ranging between 10–25° C. for a period of 1–6 hours under continuous stirring and separating the product by simple filtration and subsequently evaporation of solvents by known methods.

2. A process as claimed in claim 1 wherein the heterogeneous catalyst used is layered double hydroxide with transition metal oxides selected from a group consisting of tungstate, molybdate, vanadate and their polyanions as polyoxometalates having formula I: $[M^{II}_{(1-x)}M^{III}_{x}(OH)_2][M^{n-}]_{x/2}.zH_2O$, which is derived from LDH having formula II $[M^{II}_{(1-x)}M^{III}_{x}(OH)_2][A^{n-}]_{x/2}.zH_2O$ where M^{n-} is an anion of transition metal oxide selected from a group consisting of W, Mo, V and A^{n-} is an interstitial anion, selected from nitrate, chloride and M^{II} is a divalent cation selected from the group consisting of Mg^{2+}, Mn^{2+}, Fe^{2+}, V^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Ca^{2+} and M^{III} is a trivalent ion selected from the group consisting of Al^{3+}, Cr^{3+}, V^{3+}, Mn^{3+}, Fe^{3+} and Co^{3+}, x is the mole fraction having integral value ranging from 0.2 to 0.33, and z is the number of water molecules and ranges from 1 to 4.

3. A process as claimed in claim 1 wherein the tertiary amines, having the general formula $R^1R^2NR^3$ wherein R^1, R^2 and R^3 are the same or different and are the straight-chain or branched-chain groups selected from alkyl, alkenyl and aralkyls having C_1–C_{24} carbons selected from dimethyl decyl amine, dimethyl docyl amine, dimethylbenzylamine, cyclic amines from imidazolines pyridines, N-substituted piperazines, or N-substituted morpholines.

4. A process as claimed in claim 1 wherein the secondary amines used in the system are having general formula R^1R^2NH wherein R^1 and R^2 are the same or different and are the straight-chain or branched chain groups selected from alkyl, alkenyl and aralkyls having C_1–C_{24} carbons selected from dibutyl amine, dibenzyl amine, N-benzyl phenethylamine, N-phenyl benzylamine, cyclic amines selected from piperidine, 1,2,3,4 tetrahydro isoquinoline.

5. A process as claimed in claim 1 wherein 10–50% by weight of aqueous hydrogen peroxide is added slowly in a controlled manner during the period specified.

6. A process as claimed in claim 1 wherein the catalyst introduced in the system is 6–12 weight % anion of transition metal oxides selected from tungstate, molybdate, vanadate and their polyanions as polyoxometalates.

7. A process as claimed in claim 1 wherein the water miscible organic solvent used for the reactions is selected from the group consisting of methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol and isobutyl alcohol.

8. A process as claimed in claim 1 wherein the amount of hydrogen peroxide used ranges between 2 to 6 moles per mole of secondary or tertiary amine.

* * * * *

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United States Patent [19]

Hersh

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[54] **ANTIOXIDANT PREPARATION**

[75] Inventor: **Theodore Hersh**, Atlanta, Ga.

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[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **08/982,058**

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[51] **Int. Cl.**[6] **A61K 47/00**; A61K 9/68; A61K 9/28; A61K 9/44

[52] **U.S. Cl.** **424/439**; 424/440; 424/441; 424/464; 424/702; 514/2; 514/904

[58] **Field of Search** 424/439, 440, 424/441, 464, 702; 514/904, 2

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[57] **ABSTRACT**

A composition for reducing free radical damage induced by tobacco products and environmental pollutants. The composition includes reduced glutathione and a source of selenium. The composition can be administered orally in the form of a gel, lozenge, tablet or gum.

33 Claims, No Drawings

ANTIOXIDANT PREPARATION

TECHNICAL FIELD OF THE INVENTION

The present invention deals with the combination of several synergistic antioxidants, enzymatic co-factors and amino acids in appropriate delivery vehicles employed in solid carriers, such as tablets, lozenges and gums as a means of preventing and ameliorating signs and symptoms and complications to the oro-pharyngeal cavity and mouth including buccal mucosa, gums and tongue and the upper respiratory tract from damage caused by free radical species. Such free radical species are induced by tobacco smoke, smokeless tobacco, ingested or chewed noxious, malodorous or harmful substances and other inhaled environmental pollutants and particulate matter, including tobacco to secondary smokers.

BACKGROUND OF THE INVENTION

The deleterious effects of tobacco abuse are well known and regulatory agencies as well as the public constantly react to these scientific and epidemiologic evidences. Tobacco is indeed a worldwide public health hazard accounting for significant morbidity and mortality. Although smoking places an abundant oxidant insult to the oro-pharynx and respiratory tract, plus the local existing atmospheric pollutants in that specific environ, evidence cites the oxidant burden is on the entire organism of the smoker, particularly development or enhancement of atherosclerosis, causing cardiovascular disease, chronic obstructive pulmonary disease and various forms of cancer, including carcinomas of the mouth, pharynx, esophagus and lung.

Tobacco is a substance consisting of the dried leaves and stems of the plant *Nicotiana tabacum* which contains the drug nicotine, which is very addictive. The plant is native to North America but is now grown worldwide. Tobacco abuse has been identified as the single most preventable cause of disease, morbidity and mortality. Tobacco smoke contains many toxic chemicals and free radical species. There are three principal ways to consume tobacco: smoking, chewing and dipping and snuffing. 50 million Americans smoke and countless others are affected by tobacco smoke as secondary smokers. Children of smokers breathe this second-hand smoke and have more respiratory problems than children of non-smokers. Smokeless tobacco is used by as many as 12 million individuals and has a detrimental effect on the oral cavity plus systemic effects from buccal absorption of nicotine and other chemicals. Chewing looseleaf tobacco and "dipping" moist, ground snuff tobacco are common uses of tobacco without smoking. "Snuffing," that is, "snorting" dry powdered tobacco into the nasal passageways is rarely used in this country. Health risks from smokeless tobacco are still very significant and it is not a substitute for smoking.

Studies have estimated that tobacco smoke has over 3,000 different constituents, of which a number are toxic, some are carcinogenic and many generate free radical species. Most of these compounds have been identified in so-called mainstream and side stream tobacco smoke. The former is that volume of smoke drawn through the mouthpiece of the tobacco product during puffing while side stream smoke is that smoke emitted from the smoldering cigarette in between puffs. Although tar and nicotine are retained in the filter of cigarettes, this applies mainly to mainstream smoke, when comparing filter and non-filter cigarettes. Mainstream smoke emission is also markedly reduced both in low and in ultra low tar yield cigarettes. However, the emissions of toxic and carcinogenic components in side stream smoke are not significantly reduced in filter cigarettes when compared to non-filter counterparts. Thus, side stream smoke is a major contributor to environmental smoke, affecting both the smoker and their non-smoking counterparts, so called secondary smokers. The lower rates of consumption of cigarettes with high smoke yields has not reduced the indoor pollutants of carcinogenic substances and free radicals generating potential of tobacco smoke produced in side stream smoke, albeit their diminished levels in mainstream smoke by smoking low yield tobaccos and filter cigarettes.

Cigarette smoke induces oxidative damage to lipids, DNA and proteins, particularly protein-SH groups for this smoke contains high levels of both free radicals and aldehydes, including acetaldehyde (ethanol), propanol and acrolein, as well as other deleterious molecules.

In U.S. Pat. No. 5,060,672 (Oct. 29, 1991) which is herein incorporated by reference, Irimi and co-workers disclosed an efficient filter for tobacco smoke. Their mechanical and adsorptive filtering component also provided chemo sorptive properties to reduce aldehydes in the cigarette's smoke.

Tobacco, whether smoked as cigarettes, cigars or pipe or used as it is so-called smokeless or chewing modalities, causes common untoward effects in the oral cavity. Tobacco smoke has two chances to exert its deleterious effects in the mouth; when it is inhaled by the smoker and on its exit during exhalation. The American Lung Association states that chewing tobacco, whether one calls it snuff, a chaw, a plug, spit or smokeless tobacco is still a form of tobacco. The nicotine content is akin to cigarettes and this tobacco is etiologically responsible for oral cancer, just where it is chewed or "stored," in the mouth, cheek or gums.

Like cigarettes, evidence shows that cigars are also toxic and addictive. Cigar and cigarette smokers have a similar increased risk for oral and laryngeal cancers. While cigarette tobacco is generally flue cured with a resulting mildly acidic product, the slower curing methods for cigars render these mildly alkaline. At this pH, nicotine is more readily absorbed. Unlike cigarettes, cigars are less homogenous, and vary in size and nicotine content. Cigar smokers may spend an hour smoking a single large "Havana" although some actively inhale very little of this smoke; however, in non-inhalers, their nicotine levels may be elevated with no toxic co-absorption, as occurs in cigarette smokers. Cigar smokers also commonly hold an unlit cigar in the mouth, allowing further nicotine by local absorption. Thus, consumption of cigars may produce an equal or greater smoke burden of exposure and locally generate free radicals in the oral cavity which create deleterious effects and a risk of oro-pharyngeal disease. For cigars, as for pipe tobacco and smokeless tobacco, there is less available publicity and information for consumers than for cigarette smokers, although concomitant administration of synergistic antioxidant compositions of the present application may help prevent oral cancers and ameliorate oro-pharyngeal complications of tobacco abuse, whether from cigarettes, cigars, pipe or smokeless tobacco.

Cigarette smoke is divided into two phases, tar and gas-phase smoke. Cigarette tar contains high concentrations of free radicals. The most common oxidants include semiquinone which is in equilibrium with hydroquinones and quinones, particularly in the viscous tar matrix. Many tar extracts and the oxidants, including the latter, are water soluble and reduce oxygen to superoxide radical which can dismutate to form H_2O_2. Importantly, glass-fiber type cigarette filters retain almost all of the tar particles that are larger than 0.1 micron. Thus, the filter acts as a trap for tars in cigarette smoke. There are an inordinately large number of

free radicals, greater than 10^{15}, in each puff in the gas-phase of cigarette smoke. While the oxidants in tar are stable, those organic radicals in the gas phase smoke are reactive carbon and oxygen centered radicals with extremely short half lives. Interestingly, concentrations of free radicals are maintained at high levels for more than 10 minutes and tend to increase as tobacco smoke is aged. It is thus considered that these gas phase smoke oxidants are in a steady state as they are both continuously formed and destroyed. The latter reactions are similar to those noted to occur in smog, pointing to the extra noxious stimuli to primary and secondary smokers in polluted atmospheric environments. Although the best protection from cigarette smoke oxidant damage is cessation of smoking with personal and "environmental" abstinence, antioxidant protection is rendered by oral solutions, sprays and aerosol administration, as taught by the present disclosure, and by supplemented dietary means, as suggested by some clinical investigations. These oral sprays and inhalatory measures would ameliorate and delay putative tobacco oxidant damage in smokers and their nearby non-smoking neighbors, as well as for those who use chewing (smokeless) tobaccos.

In addition to the above, in other in vitro studies gas phase cigarette smoke was assessed in its filtered and whole (unfiltered) states for oxidative effects on human plasma. Investigators noted the prevalence of lipid peroxidation in plasma after exposure to the gas-phase smoke, but not to the whole cigarette smoke. The reaction of lipid peroxidation did not commence until the endogenous ascorbic acid had been consumed, that is, vitamin C was oxidized completely. It was noted that cigarette smoke exposure caused oxidation of plasma protein thiols (methionine and cysteine amino acid linkages) and low density lipo-proteins. It was concluded that lipid peroxidation induced by the oxidants of gas phase smoke leads to changes in the lipoproteins associated with atherogenesis. As noted in this disclosure, the synergistic effect of reduced glutathione, selenomethionine and ascorbic acid or an ascorbic acid derivative are beneficial to combating tobacco oxidants and both ameliorating and delaying the effects of tobacco smoke on the oro-pharynx and the upper respiratory mucosa.

Cells subjected to oxidative stress may severely affect cellular function and cause damage to membrane lipids, to proteins, to cytoskeletal structures and to DNA. Free radical damage to DNA has been measured as formation of single-strand breaks, double-strand breaks and chromosomal aberrations. Cells exposed to ionizing radiation and cigarette smoke have also been demonstrated to have an increased intracellular DNA damage, a precursor of mutations and development of malignancies.

Macrophage cells and neutrophils have their phagocytic activity associated with the so-called "respiratory burst" reaction, which is dependent on plasma membrane NADPH oxidase activity. The resulting oxygen radicals may then be transformed to H_2O_2 by superoxide dismutase. Investigators have shown that smokers have a higher "respiratory burst" reaction of alveolar macrophages and peripheral neutrophils than non-smokers and the former also have higher incidence of oral and respiratory signs and symptoms than non-smokers. It was determined that there is a decrease of the effect of this "respiratory burst" reaction in smokers supplemented with oral mega doses of antioxidants. The intra-oral and inhalatory preparations of the present invention with synergistic antioxidants are thus beneficial to primary and secondary smokers.

Because of the oro-pharynx's access to the environment, like the skin to oxygen and ultraviolet radiation, the structures of the oral cavity may be damaged by inhaled, ingested or chewed noxious substances and gaseous and particulate materials, especially in both active and passive smokers, as well as injuries by systemic xenobiotics and by endogenous processes, such as inflammatory reactions. Reactive oxidizing species, as induced by inhaled tobacco smoke, ozone and nitrous oxide are important factors in generating free radicals and inducing inflammatory reactions. As in other tissues, antioxidant enzymes exist in the oro-pharynx and include superoxide dismutase (SOD), which converts superoxide to hydrogen peroxide and catalase which reduces hydrogen peroxide to water. This reaction may also be catalyzed by selenium as a cofactor to the enzyme glutathione peroxidase using reduced glutathione (GSH) as a substrate. GSH-peroxidases may also reduce lipid peroxides to the corresponding alcohols also using GSH.

Glutathione, a sulphur containing tripeptide (L-gamma-glutamyl-l-cysteine-glycine) is the most abundant non-protein thiol in mammalian cells and is recognized as the primordial antioxidant. Glutathione, in its reduced form, known as GSH, acts as a substrate for the enzymes GSH-S-transferases and GSH peroxidases (with selenium cofactor) that both catalyze the reactions for the detoxification of xenobiotic compounds and for the antioxidation of reactive oxygen species and other free radicals. GSH synthesis takes place in two steps:

(1) An initial rate limiting step catalyzed by gamma glutamyl cysteine synthetase to form gamma glutamyl cysteine.

(2) Glutathione synthetase catalyzes the reaction between glycine and glutamyl cysteine to form GSH.

Intracellular stability is conferred to GSH by the gamma glutamyl bond's resistance to intracellular peptidases. This bond may be cleaved by gamma glutamyl transpeptidase which is usually located on the external surface of cell membranes. Its activity is high in the kidney, where GSH is subject to renal clearance by tubular cells and by this transpeptidation reaction, resulting in urine excretion or retransport to plasma as the constituent amino acids, glutamine, cysteine, and glycine. In this pool, along with nutritionally derived amino acids from digestion and small bowel absorption, these amino acids are available to the liver for GSH synthesis. The liver and lung also export GSH in its oxidized form denoted as GSSG, which is produced when peroxides are detoxified by GSH peroxidase. GSSG is recycled back to the reduced form, GSH, by glutathione reductase in a reaction with NADPH.

The ubiquitous glutathione plays a vital function in maintaining the integrity of the reactive oxygen species-free radical sensitive cellular components. This is accomplished through its direct role as an antioxidant, in its reduced (GSH) form, as well as a cofactor as aforementioned. In cells, GSH concentrations for antioxidant activity are maintained in equilibrium by the enzyme glutathione reductase. Under states of GSH depletion, including malnutrition and severe oxidative stress, cells may then become injured from excess free radical damage and die.

Other non-enzymatic molecules playing an antioxidant role include the ascorbates (vitamin C) which, as free radical scavengers, also react with oxidized glutathione (GSSG) and reduce it to GSH. Also, in the lipid membrane of the cells, the hydrophobic alpha-tocopherols (vitamin E) act synergistically with vitamin C to inhibit lipid peroxidation, as may be induced by cigarette smoke, by actively scavenging lipid peroxides and other radicals.

Various studies have correlated the importance of oxidant stress to various organs resulting from tobacco smoke and

other noxious environmental factors and thus continue to exert a toll on the public health of all countries. Significant morbidity and mortality result from smoking tobacco from cigarettes, cigars, and pipes and local oral pathology from chewing tobacco. Epidemiologic studies have strongly implicated tobacco in the pathogenesis of atherosclerosis and various malignancies, including oro-pharyngeal and respiratory tract neoplasias. Chronic cigarette smoking is associated with appearance of free radicals inducing oxidative damage. Measurement in blood, urine and tissues of various antioxidants or of by-products of free radical metabolic processes are supportive of tissue oxidant damage in the pathogenesis of various diseases associated with tobacco smoking and environmental pollutants.

In the oro-pharynx, cigarette smoke also accelerates the production of reactive oxygen species by recruiting local neutrophils and activation of phagocytic cells in response to the noxious agents. Attack by cigarette smoke and free radicals upon plasma proteins may be measured by carbonyl assay and by loss of enzyme activity and SH groups. Researchers have shown that whole and gas phase cigarette smoke elicit formation of carbonyl in human plasma, which is particularly inhibited by GSH. In contrast, exposure of human plasma to gas phase but not to whole cigarette smoke produces oxidative damage to lipids.

Leukoplakia, a tobacco induced white patch on the buccal mucosa, as found in smokers, is a localized irritation due to direct contact of smoked or smokeless tobacco and it is directly related to the frequency and years of tobacco abuse. Although leukoplakia is a benign oral lesion, it has a malignant potential, requiring a biopsy of the lesion to rule out cancer. Leukoplakia may regress or resolve completely when use of tobacco products is discontinued. Adequate oral examinations by primary physicians and dentists is paramount to reduce smoke induced mouth and teeth pathology.

In addition, tobacco contributes to other oral symptoms or pathologies of the mouth and teeth. Tobacco may cause halitosis, may numb the taste buds, and interfere with the smell and the taste of food. It may stain teeth and contribute to dental caries. Smokers have more dental tartar (calculus) than non-smokers. Tobacco is associated also with destructive periodontal (gum) disease and tooth loss. Acute necrotizing ulcerative gingivitis ("trench mouth") is a destructive, painful inflammatory condition occurring mainly in cigarette smokers. Swelling of the nasal and sinus membranes have also been associated, purportedly, in individuals who are "allergic" to tobacco smoke.

Besides leukoplakia, another generalized whitish hue on the buccal mucosa represents the entity of oral submucous fibrosis. This disease occurs mainly in India and is a chronic, progressive premalignant condition. The etiology is chronic chewing of tobacco or areca nut or both. The fibrosis results in restriction of mouth opening and involves the palates, tonsillar fossa, buccal mucosa and underlying muscle. Associated with this condition is also oro-pharyngeal carcinomas, also with a high frequency in India and associated in 70% of cases with chewing tobacco. Smokeless tobacco and areca nut usage is also common in Pakistan, Bangladesh and Java and in these and Indian immigrants to the United States and United Kingdom.

Over 30,000 new cases of cancer of the oral cavity are diagnosed annually, accounting for two to four percent of all new cancers. Oral cancer kills 8,000 patients each year and only half of cases diagnosed annually have a five year survival. The great majority of these patients are users of tobacco products. Other risk factors include alcohol abuse, nutritional deficiencies and poor oral hygiene.

Research has recently linked benzopyrene, as in cigarette smoke, with mutations to the human P53 gene leading to oral and respiratory malignancies. Notably, 3, 4-benzopyrene is present in polluted atmospheres of large cities such as Los Angeles, Mexico City, and London, emanating as an exhaust product of motor traffic, especially diesel engines. Breathing contaminated air with high concentrations of this compound, particularly under foggy conditions as in London, provides more than 100 times as much of this putative mutagen than for a heavy cigarette smoker. Thus, the use of the present invention as taught herein would be most beneficial to citizens, particularly if they are also smokers, of congested cities with much traffic and with smog and fog. This provides to the individual another protective measure to such free radicals and mutagens generated in their bodies, not withstanding important measures to decontaminate the atmospheric pollutants and public health and personal efforts at tobacco cessation.

Cigarette filters "trap" nicotine tars but not the gas phase compounds. Epidemiologic studies have been done in various countries to show the differential effects of tar content, amount of cigarettes smoked, type of tobacco smoked, and use of filters on oro-pharyngeal and pulmonary cancer risk in cigarette smokers.

Cigarette smoke has untold effects through free radicals and other mechanisms of affecting other organs, such as the skin. Dr. Douglas Model of England in 1985 added to the medical lexicon the term "smoker's face" from a study with pictures of 116 cases and suitable non-smoking controls. Akin to photodamage, those with smoker's face appear older and have more wrinkles. They also have a greater frequency of cancers of the lips and mouth.

Recently, sales of cigars have risen, partly due to their gaining popularity with women and the advent of the female friendly "cigar bar." Evidence, however, exists that cigar carcinogenic particles exceed those of three cigarettes and the level of carbon monoxide is 30 times greater. Fumes from cigars are of greater consequence to secondary smokers. Epidemiologic studies reveal greater frequencies of heart disease, emphysema, and cancers of the mouth and pharynx in cigar smokers when compared to matched non-smokers.

There are a number of preparations on the market as dentifrices, gels, breath fresheners and mouthwashes and oral rinses to protect the mouth and teeth from the effects of chewed or smoked tobacco. Cigarette tar may deposit on the teeth, gums, tongue and other surfaces of the oral cavity of smokers. Tobacco tar, a dark, oily, viscid blend of polycyclic aromatic and aliphatic hydrocarbons, is produced in cigarettes, cigars, or pipe smoke by the burning of the tobacco. The smoker inhales the tar and other tobacco smoke combustion products are sucked into the oral cavity and respiratory passages. The smoke is then exhaled, passing a second time through the mouth of the smoker, anew depositing tar. This causes discoloration of the teeth and other oral surfaces. Not only may there be smoker's "bad breath" but also tooth decay and gum disease. Smokeless tobacco is equally locally deleterious. Food particles, oils and other substances may also be deposited on mouth surfaces. The tars and mainstream smoke will elicit free radical and inflammatory responses in the mouth and other mucosal surfaces. The antioxidants and reparative preparations of this invention may be prepared as oral and dental compositions as well as with optional added ingredients that are also breath fresheners, fluorides, anti-microbials, and solubilizers of tars and essential oils. Most of the dental products used as "anti-tobacco" are in the form of toothpastes and gels.

Diamond patented a combination of non-ionic and anionic surfactants with at least one essential oil as dental and oral preparations for smokers for solubilizing and removing tobacco tars as well as onion and garlic essential oils. U.S. Pat. No. 5,514,366 (May 7, 1996), herein incorporated by reference, teaches complimentary uses of the preventive and reparative effects of the present invention.

GSH has been shown to have multiple functions in detoxification and its depletion in extracellular fluids and cells is associated with an increased risk of chemical toxicity. Although there are large variations in dietary sulphur amino acid content, these variations do not correlate with GSH levels in the blood plasma pool. These GSH levels, however, do vary with age, race and gender of human subject and with dietary habits and intakes. Investigators have reported that extracellular pools of GSH, including plasma, respiratory tract lining fluid and oral and small intestinal lumen are GSH vital protectants against chemically induced injury. These would include the chemicals in tobacco smoke and other environmental pollutants as well as chemicals in smokeless tobacco preparations and other chewable or orally ingested substances. The aforementioned pools, through GSH and related synergistic antioxidants, as proposed in the present invention, detoxify chemicals extracellularly, supply GSH and its precursor amino acids to cells and protect the extracellular surface of the plasma membrane from damage. Alterations in GSH status could thus alter this regulatory function by GSH and thereby lower the threshold for chemically induced cell death by apoptosis, making GSH both a useful protectant to and biomarker for risk from a variety of single or mixtures of deleterious chemicals, such as in various types of tobacco.

Some mammalian cells are able to absorb intact the tripeptide glutathione. It may also be synthesized by some organs, particularly the liver. Various scientific papers have addressed a method for proper replacement of glutathione, particularly to increase cellular levels in glutathione depleted states. Certain diseases cause glutathione depletion from interaction endogenously with metabolic intermediates, the various deleterious free radical species. Labeling glutathione, at the intracellular level as the "antidote physiologically appointed to the neutralization and thus detoxification, by the formation of covalent bonds, of highly reactive toxic substances of endogenous or exogenous origin," Pilotto and coworkers patented dipeptide compounds with pharmaceutical properties to replete the body's glutathione levels. Their U.S. Pat. No. 4,761,399 dated Aug. 2, 1988, teaches raising glutathione levels by various routes, including oral, inhalation and parenteral methodologies. Meister, in U.S. Pat. No. 4,710,489 issued Dec. 1, 1987 teaches new molecules to increase cellular levels of glutathione. The invention of the '489 patent deals with using pure alkyl mono-esters of glutathione, wherein the ester is a glycine carboxylic acid. These molecules may be administered orally or by injection.

It is thus an object of the present invention to provide various compositions and methods of employing such compositions for preventing and ameliorating signs and symptoms and complications to the oro-pharyngeal cavity and mouth including buccal mucosa, gums, teeth and tongue as well as the upper respiratory tract as a result of tobacco oxidants and other gaseous and particulate matter pollutants.

These and further objects will be more readily appreciated when considering the following disclosure and appended claims.

SUMMARY OF THE INVENTION

The present invention is directed to a composition of synergistic antioxidants and the use of this composition employed in a gum, tablet or lozenge delivery systems to prevent and ameliorate free radical damage induced by smoking and environmental pollutants to the structures of the oro-pharynx and upper respiratory tract. Active ingredients include reduced glutathione, selenium as an element or as a seleno amino acid like selenomethionine and optionally the sulphur-containing amino acids L-cysteine and N-acetyl-L cysteine and/or L-methionine. As further optional ingredients, the composition contemplates the use of ascorbic acid and/or its derivatives, alpha-tocopherols and/or its derivatives. Depending upon the target organ and additional disease states or conditions encountered, other anti-oxidants or molecules may be included in these preparations. These include, but are not limited to, the enzyme superoxide dismutase, vitamin A and/or beta-carotene, zinc and fluoride compounds.

DETAILED DESCRIPTION OF THE INVENTION

Antioxidants have been found to inhibit all stages of carcinogenesis whereas other antioxidants are more specific and thus more effective against tumor initiation or promotion or tumor progression. Glutathione and selenium have been shown to play prime roles in protection of carcinogenesis and also in preventing other cancers, when selenium is taken orally thereby replenishing selenium body stores.

Likewise, glutathione, inhibits carcinogenesis, and indeed when its concentration is suppressed by chemicals so that glutathione levels are significantly lowered, chemical carcinogenesis is enhanced and progression of tumor numbers and tumor size increases. Reducing the intracellular levels of GSH in cells increases their sensitivity to oxidant damage. Studies have shown that increases in intracellular GSH are beneficial. An L-cysteine delivery agent not only enhanced endothelial cell GSH concentration, but also protected these cells from damage from endogenous hydrogen peroxide. This preventive role of GSH is of significant biologic value. Without being bound to any particular theory, it is noted that reduced glutathione is employed in protecting cells against oxidative stress by itself being oxidized. Thus, L-glutathione must act in combination with other enzyme systems in order to be reduced so that it may renew its role as a free radical scavenger. GSH functions also coordinately with the enzyme glutathione peroxidase which requires selenium as a cofactor to exert its biologic antioxidant function. Selenium compounds have been shown to scavenge oxygen-centered radicals in vivo with reduced glutathione through glutathione peroxidase. It is believed that selenium-GSH peroxidase catalyzes toxic hydrogen peroxidase in the presence of reduced glutathione. This reaction reduces glutathione to oxidized glutathione GSSG. In turn, the GSSG is reduced back to GSH by the enzyme GSH reductase thereby maintaining abundant cellular GSH to scavenge free radicals anew.

In summary, the major functions of reduced glutathione (GSH) in protection against lipid peroxidation are related to three types of reactions, all inter-related and synergistic in combining non-enzymic scavengers and enzymic and dietary provided antioxidants.

1. GSH with selenium co-factor glutathione peroxidases eliminate toxic peroxides.
2. GSH reduces oxidized forms of vitamin C which, in turn, maintains vitamin E in its reduced form promoting its metabolic functions. Thus, GSH supports the free radical reductions and free radical chain-terminating functions of the two nutrient antioxidants, vitamins C and E.

3. GSH functions through glutathione S-transferases to detoxify reactive aldehydes created during the process of lipid peroxidation.

As noted too, some cells have sodium dependent up-take systems for GSH, allowing cells to use both exogenous GSH and endogenously synthesized GSH, thereby enhancing a cell's ability to survive oxidative and free radical species damage. In this fashion, extra-cellular GSH also protects cells' survival.

Investigative studies have shown that cells' viability correlates best with content of GSH in mitochondria. In the absence of GSH, lipid peroxidation is uncontrolled and leads to cell injury and death. Conversely, GSH protects cells from the ravages of free radicals, working synergistically with the antioxidant enzymes and the dietary vitamin antioxidants.

For those in the chicle or gum industry, the compositions of the base for both sugar containing chicles and reduced calorie chewing gums and bubble gums are well known. The present invention can be used with reduced calorie gums, particularly with the polyols xylitol and sorbitol, but this invention is not restricted to these sweeteners. These chewing gums generally contains 20–30% of a water insoluble gum base, and from 30 to 90% of a filler or texturizing agent (called bulking products). Water soluble flavorings are also added. The gum base may also contain plasticizers or softeners to improve the consistency and texture of the gum. Some of these chicles are saliva stimulating chewing compositions with specific salivary stimulants. One such composition is taught by Cherukuri et al. in U.S. Pat. No. 4,980,177 dated Dec. 25, 1990, which is herein incorporated by reference.

Another method for the application of the active ingredients of this invention in the chewing gums, bubble gums, lozenges and tablets is to incorporate the various antioxidants, minerals and amino acids in liposomes or other state of the art encapsulating vehicles, akin to nanospheres, glycospheres and others as used in topical compositions. Liposomes are lecithin spheres that form an oil protective membrane around the putative active ingredients of these compositions. These carriers also deliver the active ingredients locally for their preventive and therapeutic functions as well as systemically through buccal mucosal absorption. Unger and co-workers have taught therapeutic drug delivery systems comprising gas-filled liposomes which encapsulate the active preparation in U.S. Pat. No. 5,580,575 dated Dec. 3, 1996, which is herein incorporated by reference. Earlier, Chakrabarti et al. and U.S. Pat. No. 5,5380,531 dated Jan. 10, 1995 which is also herein incorporated by reference, disclosed preparations comprising a lipid and a modified peptide for encapsulating amino acids into liposomes.

As noted above, xylitol is contemplated for use herein as a sweetener to mask the taste of the present active ingredients. Xylitol chewing gums have been evaluated in various field studies for their ability to influence the rates of dental caries in children. Makinen and co-workers did a 40 month double blind cohort study of nine treatment groups, including various doses of xylitol, on the relationship between the use of these chewing gums and the development of cavities. The study was performed in 1989–1993 in Belize on 1,277 children. They showed significant reductions in rates of dental caries in xylitol treated groups, compared with the no-gum chewing group and concluded that the systematic usage of polyol based chewing gums reduces the frequency of cavities, with xylitol chicles being even more effective than sorbitol gums. Other studies have also revealed that high content xylitol confections, including candies and chewing gums are not only non-cariogenic but also inhibit caries.

As a further preferred embodiment, the current application contemplates coating chewing gums with at least some active ingredients to prevent dental caries and dental plaque, like the aforementioned xylitol sweetener and flavors plus anti-halitosis compounds, line zinc salts, to act as breath fresheners and to combat oral malodor. The synergistic complex of antioxidants will be paramount and include, as stated, at least approximately 0.5 mg L-glutathione, 5 mcgm selenium as selenomethionine and optionally 15 mg vitamin C and 10 IU vitamin E to neutralize and scavenge free radicals in the oral cavity. Other optional ingredients include superoxide dismutase, vitamin A, beta-carotene, the amino acids cysteine, methionine, taurine, and/or arginine, as well as zinc salts, such as zinc acetate or zinc gluconate. The substances incorporated in the gum are released in the mouth to exert their beneficial effect during the process of mastication. The active ingredients may act locally and may also be absorbed through the buccal mucosa for systemic use.

Hill in the '530 patent summarizes referenced patents which deal with chewing gums which provide flavors and which deliver active substances into the oral cavity. For example, U.S. Pat. No. 3,075,884 teaches the mixing of active ingredients in corn syrup which is coated unto a gum. U.S. Pat. No. 3,011,919 teaches a method for incorporating actives by providing coatings with wet sugar. U.S. Pat. No. 3,352,689 discloses formulations of a sugarless gum, also to release actives in the mouth.

Hill's '530 patent also discloses a number of references dealing with chewing gum compositions containing anti-plaque properties. In addition, Hill also teaches the use of xylitol and sorbitol in chewing gums.

Westall et al in U.S. Pat. No. 3,821,417 dated Jun. 28, 1974, which is also herein incorporated by reference, discloses the use of dihydrochalcone as well as the use of various antioxidants in chewing gums, namely butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate.

Although the chewing gums and bubble gums of this invention will preferentially be reduced-caloric compositions, the present invention may optionally include compositions with metabolizable sugars. These chewing gums compositions are well known in the industry and can include a gum base (about 40% to 60% by weight of the composition), which comprise an elastomer, a polyvinyl acetate polymer, an acetylated monoglyceride, a wax with melting point below 60 degrees C., an elastomer solvent, plasticizer and a filler. The gum is then provided with the present synergistic antioxidant complex so that each piece of gum has approximately at least 0.1 mg L-glutathione, 2.0 mcgm selenium as selenomethionine, 10 mg ascorbic acid, 2.5 IU vitamin E and 1.0 mg L-cysteine or 1.5 mg N-acetyl cysteine. It is noted that the reduced calorie gum could contain xylitol or lactitol as the sweetener while the standard calorie gum will have sucrose, lactose or other mono-or di-sacharides, plus flavoring agents. Chewing gums may in addition contain saliva stimulating compounds, usually organic acids, such as described by Cherukuri et al. in their '177 patent.

As optional embodiments, other ingredients may be added such as breath fresheners and breath cleansing (anti-halitosis) agents. Plevy taught in U.S. Pat. No. 4,740,368 dated Apr. 26, 1988, which is herein incorporated by reference, compositions with amylase as breath cleansing confections. Alpha amylases are synthesized by the salivary glands and exocrine pancreas and are able to digest carbohydrates. Plevy's preparation used 1–8-skb units of alpha-amylase of fungal origin to degrade starch. Along with

artificial sweeteners and flavoring, this enzyme is the main ingredient of comestible confectionary bases, such as gums and lozenges. Separately, Pera in U.S. Pat. No. 4,775,525 dated Oct. 4, 1988 which is also herein incorporated by reference teaches a dental formulation containing sodium alginate. It is used as a calcium chelating agent which weakens the bond between the plaque and the teeth. The referenced patent advocates the concomitant use of benzalkonium chloride and zinc.

In addition to providing these synergistic anti-oxidants in standard chewing gums, alternatives to chicle gum is contemplated. Conventional chewing gums are not digestible or biodegradable and cause on disposal unsightly litter. Thus, gum base substitutes which are edible have been the object of various teachings. For example, U.S. Pat. No. 5,366,740 by J. J. Shaw dated Nov. 22, 1994, which is herein incorporated, uses a wheat gluten preparation as an edible "chewing gum". Its manufacture includes calcium carbonate, glutinous rice flour, and ascorbic acid as softeners of the wheat gluten, as well as other ingredients commonly used as wheat flour dough conditioners. If used herein, such a chewable gum would also provide the present synergistic anti-oxidants application to the oral mucosa to combat tobacco smoke, chewing tobacco or other environmental ingested or inhaled pollutants which all induce free radicals. The edible gum may also have breath fresheners. All the components of this chewable gum, distinct from commercially available "chewable tablets" may then be swallowed while its protein and carbohydrate bases are digested. All residual vitamins, minerals, and amino acids not absorbed by the buccal mucosa during the process of chewing will be swallowed and then made available systemically to the body following their intestinal absorption. This would be akin to the aforementioned constituents in conventional chewing gums which are swallowed dissolved or dispersed in saliva.

When the present invention is in the form of gels, lozenges, gums, candy, chewable tablets, or chewing gums, flavoring may be added. Flavors may be based on oils of spearmint and peppermint. Other flavoring materials may include menthol, clove, cinnamon, wintergreen, citrus fruits, eucalyptus, aniseed and others which are commercially available. Flavors may range in concentrations depending on the product from about 0.1 to about six percent by weight of the total composition.

When the products are in a form of gels, bicarbonates may be present in the composition with thickening agents, in a concentration from 0.5 to 5.0% by weight. State of the art thickeners with a bicarbonate and zinc salts, include, but are not limited to chicle, xanthan, arabic, karaya or tragacanth gums. Alginates, carrageenans and cellulose derivatives such as sodium carboxymethyl, methyl, or hydroxyethyl compounds can also be included, as well as surfactants and abrasives. In order to decrease dental cavities and add flavor, without using metabolizable sugars, sweetening agents as saccharin, sodium cyclamate, sorbitol, aspartamane, and others may be used in concentrations from 0.005 to 5.0 per cent weight of the total composition, although, as stated xylitol is the preferred sweetener.

Researchers in Finland showed that chewing gum containing the natural sweetener xylitol reduced chronic ear infections in children. Xylitol, a five carbon sugar alcohol, found in birch and maple trees and corncobs, also has been used to fight tooth decay, since local mouth bacteria cannot digest this sweetener. It appears that xylitol prevents bacteria from attaching to cells in the posterior area of the mouth from whence they could enter the ear passages and cause infection. Xylitol is of value for oral hygiene since it is not metabolized by mouth bacteria so that organic acids which attack teeth are not produced. Xylitol reduces dental caries and reduces the amount of plaque-forming bacteria (streptococcus mutans) in the mouth. Various clinical studies in other countries have confirmed the unique dental benefits of xylitol, thus it is of particular use in pediatric mellitus. Therefore, xylitol preferably will be used as a sweetener in these compositions, particularly in the chewing gums and lozenges of the present invention for its known noncaloric, salutory oral and dental effects.

Lactitol may also be used as a substitute for sucrose in sugar-free compositions of the present invention. Lactitol is a disaccharide sugar alcohol derived from lactose, highly water soluble and of low hygroscopicity, making it a suitable non-caloric sweetener for use in tablets and other solid dosage forms.

A number of compounds may be added to the present chewable tablets, lozenges, candies and gums in order to enhance their aromas or tastes. These substances may also impart fragrances to the aforementioned. Grapefruit and citrusy aroma and flavors have been included in smoking tobacco articles both prior to smoking and on smoking in both the main stream and the side stream smoke. Methyl phenyl pentanol derivatives have been used to augment and enhance aromas, such as in perfumes and colognes. Schreck patented these derivatives for use in tobaccos and tobacco articles as taught in U.S. Pat. No. 4,458,699 dated Jul. 10, 1994, which is herein incorporated by reference. Floral, green, weedy, fruity, minty, citrusy, oriental and green-pepper-like aroma and taste nuances are well known to those skilled in the art of flavors and fragrances which can be used in the present oral compositions including chewing gums, bubble gums, chewable tablets, lozenges and candy.

Flavored hard tablet and lozenges prepared pursuant to the present invention are made with the recommendation that the user dissolve them so that the ingredients are both delivered to the oral cavity and remaining molecules, not absorbed by the buccal mucosa, will be admixed with saliva and swallowed normally. Candy in the form of tablets with various flavors and the sweetener xylitol are manufactured using state of the art technology as known in the confectionary industry. These tablets are intended to carry the above described synergistic complex of antioxidants. For example, one such tablet can have the following ingredients in amounts as recited:

4 (four) tablets equal the following dosage per day:

L-Glutathione	40 mg.
Selenomethionine	25 mcgm
Ascorbic Acid (Vitamin C)	30 mg.
Alpha Tocopherol (Vitamin E)	15 IU
Retinyl Acetate (Vitamin A)	2500 IU
L-Cysteine	10 mg.

Lozenges may be "flavored" with standard therapeutic agents such as methol, eucalyptus, and ingredients known in the "cold products" industry.

The components of the present synergistic antioxidant complex may also be incorporated into chewable flavored tablets. Such chewable tablets may be enhanced with sugars like sucrose, fructose and/or lactose. Alternatively, artificial sweeteners, such as xylitol, lactitol, sorbitol and the like can be used herein. Natural flavors such as citrus fruits, cherry, strawberry, grape, and the various mint flavors, to name a few, can be incorporated in chewable tablets of the present invention. Inactive ingredients, as vehicles for these flavorings include dextrins, starch, silica, gelatin, hydrogels, mag-

nesium stearate and phosphate, glycerides of stearic and palmitic acids, and usual fillers and thickeners as commonly employed in chewable tablets and vitamins, such as are commercially available.

Serving sizes of these chewable tablets may vary so that a consumer can ingest from one to four chewable tablets per day. The active ingredients contained within the tablet can be varied so that in consuming the recommended number of tablets, usually from one to four, the user will ingest the recommended or minimum daily requirement of the active ingredient as prescribed by dietary supplement guidelines.

For example, a chewable tablet designed as a two a day dosage (morning and evening) would have the following active ingredients:

L-Glutathione	20 mg.
Selenomethione	25 mcgm
Ascorbic Acid (Vitamin C)	30 mg.
Alpha Tocopherol (Vitamin E)	15 IU
Retinyl Acetate (Vitamin A)	2500 IU
L-Cysteine	10 mg.

To this tablet, one could also include xylitol or lactitol as the sweetener with flavorings according to taste.

In a most preferred aspect of the present invention, the aforementioned oral or inhaled pharmaceuticals, amino acids and active antioxidant containing composition has a formulation for total daily consumption to include recommended daily allowances of reduced L-ascorbic acid, tocopherols, and other vitamins. In addition to L-glutathione, the preferred selenium dosage is approximately at least 10 mcgm of elemental selenium per day most preferably 25 mcgm per day. This may also be used as selenomethionine, which is commercially available in a 0.5% trituration with dibasic calcium phosphate. This fine powder contains from 5,000 to 5,300 mcgm of selenium per gram of the selenomethionine preparation. The compositions may also have about 30 IU of D,L-alpha tocopherol and about 1000 mcgm of vitamin A, as the retinol equivalent or 5,000 units as vitamin A with a range of 20–40% beta carotene. These are recommended daily allowances and these active ingredients may be administered in oral liposomes, either each encapsulated alone or in combinations. Knight and co-workers in U.S. Pat. No. 5,049,388 (Sep. 17, 1991), incorporated herein by reference, disclosed small particle aerosol liposomes. These particles had diameters less than 5 microns. Medications were combined with the liposomes such that the drug or active ingredient interacted with the liposome membrane.

The aforementioned compositions may be particularly useful in the prevention and treatment of tobacco smoke or other gaseous or particulate matter exposure, including buccal damage from chewing tobacco. They represent a delicate balance of ingredients which serve not only to reduce the number of free radicals but also to inhibit the tissular metabolic oxidation. The more preferred formulations in accordance with the present invention also enhance the performance of the composition by recycling certain antioxidant ingredients in the formulation after these are absorbed and by offering the formulation allowing for long term use. These compositions when provided in sufficient dosage over a period of time may be useful in the treatment and the prevention of the damage caused in the oropharynx and upper respiratory tract, by exposure to tobacco smoke, smokeless tobacco and other environmental pollutants.

Glutathione and selenium act synergistically in vivo as they are both constituents of the same enzymatic system. GSH serves as a specific donor substrate while selenium, provided from alimentary sources or locally from topically administered preparations of selenium, selenoamino acids or selenium yeast extract, provides the prosthetic group of GSH peroxidase. The glutathione and selenium antioxidant functions are intrinsically related since by keeping a peroxidase in action, the GSH and selenium, contribute to the removal of the dismutation product of free oxygen radicals, namely, hydrogen peroxide. In a broad sense, GSH and selenium modulate free radical chains initiated or sustained by hydroperoxides. Selenium is used in the present invention for its role as an antioxidant as well as its anticarcinogenic and antimutagenic properties. Thus, selenium-glutathione complex may lower the level of potentially damaging peroxide radicals that are generated from various carcinogenic promoting chemicals, including tar phase and gas phase tobacco smoke inhaled by-products, particularly side stream smoke.

Glutathione peroxidase, a group of water soluble enzymes, also catalyze the destruction of both aqueous and membrane-bound hydroperoxides. In dietary selenium deficiency, these enzyme levels are markedly decreased resulting in severe free radical damage to the tissues so involved. The other related antioxidant systems cannot make up for depressed local activity of selenium and selenium dependent enzymes. Thus, the importance of providing selenium in these intra-oral antioxidant preparations, as well as ascertaining adequate nutritional supplements. Selenium may be provided as a selenoamino acid, like selenomethionine, as such, is protected in oral liposomes.

L-ascorbic acid (vitamin C) or its derivatives can be employed in these compositions primarily for their antioxidant activities. Stabilized vitamin C is employed so that it does not lose its physiological reducing activities because of its high susceptibility to oxidation. The minimum daily requirement for adults has been established. It appears, however, that cigarette smokers need supplemental vitamin C. Vitamin C, as an antioxidant, has been employed in vitamins, beverages, foodstuffs, pharmaceuticals and cosmetic preparations. Vitamin C has also been used for the prevention of viral diseases and a preventative by its antioxidant properties of development of cutaneous premalignant lesions and malignant tumors. Sakai, et al in U.S. Pat. No. 5,508,390 (Apr. 16, 1996) have outlined uses of an L-ascorbic acid. Such stabilized vitamin C is used as an additive in various preparations. The emphasis of this preparation of ascorbic acid is in its stabilizing and reducing function. Todd, Jr., in U.S. Pat. No. 5,084,293 (Jan. 28, 1992), describes a method of using "activated" ascorbic acid preparations with antioxidant compositions. These include anhydrous compositions to embody propylene glycol or non-ionic surface-active agents to provide vitamin C with increased antioxidant activity in fats, oils, and carotenoids.

Vitamin C, ascorbic acid, plays a major role in human metabolism. As an antioxidant, it protects the skin from free radical damage induced by radiation, tobacco smoke, and other inhaled or swallowed environmental pollutants. Vitamin C promotes collagen synthesis, tissue repair and wound healing. Vitamin C also renders important protection against damaging chemicals associated with cigarette smoking, including nicotine, carbon monoxide, nitrogen oxides, nitric acid gas and others. Although ascorbic acid may be reduced in this scavenging role, the ascorbate radical may then be removed by the NaDPH enzyme systems as sources of reducing molecules. Thus, vitamin C may be recycled to abate or lessen the process of lipid peroxidation by its synergistic function with others. Markham in U.S. Pat. No.

4,822,891 refers to the oral administration of vitamin C to demonstrate its free radical attributes. Others have shown that chronic tobacco smokers had higher urinary levels of 8-EPI-prostaglandin F2A than non-smokers. Oral supplementation with vitamin C suppressed urinary levels of this metabolite, suggesting a reduction of oxidant stress in these subjects.

Cigarette smokers often have lower plasma levels of ascorbic acid than matched non-smoking controls. Clinical and investigative evidence suggests that smokers may have a higher ascorbic acid requirement and that supplementing dietary vitamin C may be protective to the smoker. In vitro studies have shown that antioxidants and reducing substances may prevent the removal of elastase inhibitor capacity induced by cigarette smoke.

Other components were also investigated as being useful in practicing this invention, for example, the sulphur containing amino acid cysteine is one of the three amino acid constituents of the tripeptide antioxidant glutathione. Studies have shown that cysteine and cysteine derivatives, as recited in U.S. Pat. No. 4,910,222 by Puricelli, have liquefying and expectorating properties. These compounds may be administered by the oral route as a solid (capsules), or as a liquid (emulsions) and by aerosol sprays.

Vitamin A is an essential nutrient. Relative vitamin A deficiency may adversely affect the skin and mucous membranes, including the mucosa of the oral cavity. These alterations are reversible on repletion with vitamin A or one of its derivatives.

Xylitol, the sweetest of all bulk sugar substitutes, tastes like sugar, leaves no aftertaste, and has 40% less calories than sugar. A caloric value of 2.4 kcal/gram is accepted for nutritional labeling. This five carbon sugar alcohol has a negative heat of solution which causes a cooling effect when it dissolves in the mouth, particularly concomitantly using mint flavors. When sugars are ingested, the micro-organisms in the mouth ferment the sugars with a consequent drop in pH, even to a low pH of 4. When the contents in the mouth are acid, there is demineralization of tooth enamel. Stimulated flow of alkaline saliva is then necessary to return the pH in the mouth to normal levels. Thus, use of xylitol reduces susceptibility to dental caries by helping to remineralize affected teeth and by inhibiting demineralization of healthy teeth. Studies have also shown that adults chewing xylitol gum or xylitol/sorbitol gums or mouth rinses develop significantly less dental plaque than controls chewing sucrose gums. Their dental plaque too showed an improvement in the ability to resist any drop in pH. In the xylitol groups, studies show that xylitol inhibits the growth of various oral cariogenic bacteria, particularly those of Streptococcus mutans.

Although the susceptibility to dental caries is influenced by various factors including diet and eating patterns, tooth surface and salivary rates are also important. The resistance of the enamel and dentine may be increased by regular exposure of the teeth to fluorides systemically. This is accomplished via drinking water and fluoride tablets or topically via toothpaste, gels and mouthwash. Various studies have demonstrated that consumption of even small quantities of xylitol enhances the beneficial effects of existing fluoridation program, resulting in reduction in new caries. Similar trends have been observed when xylitol is applied topically such as a mouth rinse.

When the present preparations are in the form of gums, tablets or lozenges, flavorings may be added to these compositions, as per the state of the art in these respective industries. Flavors may be based on oils of spearmint and peppermint. Other flavoring materials may include menthol, clove, cinnamon, wintergreen, citrus fruits, eucalyptus, aniseed and others commercially available for these flavoring purposes.

Flavors may range in concentrations depending on the product from about 0.1 to about 6.0% by weight of the total composition.

When the products are in a form of gel, bicarbonates may be present in the composition with thickening agents, in a concentration from 0.5 to 5.0% by weight. State of the art thickeners with bicarbonate and zinc salts include, but are not limited to chicle, xanthan, arabic, karaya or tragacanth gums. Alginates, carrageenans and cellulose derivatives such as sodium carboxymethyl, methyl, or hydroxy ethyl compounds as appropriate for the intended preparations, surfactants and abrasives may also be included. Alcohols will otherwise be avoided for their known risk factor for oral cancers. In order to decrease dental cavities and add flavor, without using metabolizable sugars, sweetening agents as saccharin, sodium cyclamate, sorbitol, aspartamane and others may be used in concentrations from 0.005 to 5.0% by weight of the total composition, albeit xylitol, vide supra, is preferred.

Lactitol may also be used as a substitute for sucrose in our sugar-free compositions. Lactitol is a disaccharide sugar alcohol derived from lactose, highly water soluble and of low hygroscopicity, making it a suitable non-caloric sweetener for use in solid dosage forms.

A number of compounds may be added to the various liquid compositions of this invention in order to enhance the aromas or tastes of these preparations. These substances may also impart fragrances to the aforementioned. Grapefruit and citrus aroma and flavors have been included in smoking tobacco articles both prior to smoking and on smoking in both the main stream and the side stream smoke. Methyl phenyl pentanol derivatives have been used to augment and enhance aromas, such as in perfumes and colognes. Schreck patented these derivatives for use in tobaccos and tobacco articles in U.S. Pat. No. 4,458,699 (Jul. 10, 1994) which is herein incorporated by reference. Floral, green, weedy, fruity, minty, citrusy, oriental and green pepper-like aroma and taste nuances are well known to those skilled in the art of flavors and fragrances for such compositions as in oral sprays, mouthwashes, mouth rinses, gels, dentifrices and other medicinal, nutritional or breath freshener products.

Wahl and co-workers at the National Institutes of Health taught methods to treat chronic inflammatory diseases in U.S. Pat. No. 5,499,688 (Sep. 12, 1995), which is herein incorporated by reference. They administered effective amounts of nitric oxide scavengers to decrease the amount of putative nitric oxide present at the site of the inflammation. These compounds belonged to complexes with L-arginine, L-canavanine, citrulline and amino guanidine. They note, akin to the argument herein favoring the use of antioxidants to neutralize free radicals. This '688 patent augurs a method for treating gingivitis and periodontitis. Kleinberg in U.S. Pat. No. RE31181 (Mar. 15, 1983), which is also herein incorporated by reference, also teaches arginine and arginine peptides for oral care preparations.

Over the centuries, Chinese herbalists have identified individual herbs that have either beneficial effects on the human body or even therapeutic properties. The National Institute of Health has recently established an agency for research in these so-called alternative therapies.

It is preferred that the antioxidants of the present invention be provided in a form which is as pure as possible. They should be present without noxious lubricants (sand, soaps,

talc), fillers, colors, binders, dispersants or like adjuvants sometimes employed as delivery excipients in the aerosol pharmaceutical industry.

Various products may be administered to reduce the viscosity of mucin in sputum. Productive cough is a common symptom. Mucus in the respiratory tract, especially in chronic tobacco smokers as well as other conditions including cystic fibrosis, may be treated with cough syrups and expectorants. Ceramin and Tabachnik described the use of reducing sulfhydryl compounds to decrease the sputum viscosity in patients with pneumonia, chronic bronchitis and cystic fibrosis in U.S. Pat. No. 4,424,216 (January, 1984), which is herein incorporated by reference. They proposed as the preferred sulfhydryl agent WR 2721 to be given orally in capsules of gelatin so that in vivo it may then release free sulfhydryl groups.

Cough suppressants, bronchodilators as well as agents like cysteine, arginine, methionine, taurine, vitamin A and others to reduce mucin viscosity may be added to these compositions. The latter will make sputum more liquid and easier to expectorate for these patients and for chronic tobacco abusers. U.S. Pat. No. 4,927,850, herein incorporated by reference, described methionine in oral or parenteral preparations for ameliorating inflammatory symptoms of respiratory diseases.

As an optional embodiment, the compositions herein described with synergistic antioxidants to combat free radicals in the oropharynx may also contain zinc or zinc compounds. The state of the art of oral care and hygiene has long recognized the value of zinc to neutralize oral malodor and the value of zinc ions for their anti-plaque and anti-calculus properties. Mouth rinses, mouthwashes, gels and dentifrices will thus complement the properties of the xylitol sweetener in oral and dental preventative care.

Various patents have described different zinc compounds and other complexes in oral compositions. Domke and Bergman taught an aqueous zinc-polyamide complex as a solution for control of halitosis, dental care and to decrease the astringency and metallic taste of zinc in the mouth in U.S. Pat. No. 5,587,147 (Dec. 24, 1996), which is herein incorporated by reference. This patent discloses previous documents dealing with zinc salts such as zinc chloride, zinc phenol sulfonate, zinc citrate and other zinc complexes, some of which purportedly also exhibit oral antimicrobial activities. The zinc ion concentrations in these compositions will be at least 0.1 to 3.0 weight percent and these will preferably be in an alkaline pH to avoid demineralization of tooth enamel at acid levels. In any event, these aqueous compositions will not have a pH below 6, and preferably about 7.

Another method of application of the active ingredients in the gum, tablet or lozenge products of the present invention is to incorporate the various anti-oxidants, minerals and amino acids in liposomes or other state of the art encapsulating vehicles, akin to nanospheres, glycospheres and others as used also in topical compositions. Liposomes are lecithin spheres that form an oil protective membrane around the putative active ingredients of the composition. These carriers also deliver the active ingredients locally for their preventive and therapeutic functions as well as systemically through buccal mucosal absorption. Unger and co-workers, in U.S. Pat. No. 5,580,575 (Dec. 3, 1996), which is herein incorporated by reference, have taught therapeutic drug delivery systems comprising gas-filled liposomes which encapsulate the active preparation. Earlier, Chakrabarti and associates, in U.S. Pat. No. 5,380,531 (Jan. 10, 1995), which is also herein incorporated by reference, disclosed preparations comprising a lipid and a modified peptide for similar uses of amino acids and peptides into liposomes. Knight et al. (U.S. Pat. No. '388) has taught about small particle aerosol liposomes and liposome combinations for medical delivery uses.

I claim:

1. A composition for reducing free radical damage induced by tobacco products and environmental pollutants comprising, as active ingredients, reduced glutathione and a source of selenium selected from the group consisting of elemental selenium, selenomethionine and selenocysteine, said active ingredients being combined with suitable carriers and flavorings for their intra-oral administration as gels, lozenges, tablets and gums in concentrations for reducing free radical damage induced by tobacco products and other environmental pollutants to the oral cavity, pharynx and upper respiratory tract of a user and secondary smokers.

2. The composition of claim 1 wherein each of said gels, lozenges, tablets and gums contains at least approximately 0.5 mg. of said reduced glutathione.

3. The composition of claim 1 wherein said gels, lozenges, tablets and gums contains at least approximately 5 mcgm. of said source of selenium.

4. The composition of claim 1 wherein said gels, lozenges, tablets and gums further contain at least approximately 15 mg of vitamin C as ascorbic acid or as a derivative of ascorbic acid.

5. The composition of claim 1 wherein said gels, lozenges, tablets and gums further contain at least approximately 10 IU of vitamin E as alpha tocopherol.

6. The composition of claim 1 wherein said gels, lozenges, tablets and gums further contain superoxide dismutase.

7. The composition of claim 1 wherein said gels, lozenges, tablets and gums further contain vitamin A.

8. The composition of claim 1 wherein said gels, lozenges, tablets and gums further contain beta carotene.

9. The composition of claim 1 wherein said gels, lozenges, tablets and gums further contain at least one amino acid selected from the group consisting of cysteine, methionine, taurine and arginine.

10. The composition of claim 1 wherein said gels, lozenges, tablets and gums further contain a zinc salt.

11. The composition of claim 10 wherein said zinc salt comprises zinc glutonate.

12. A gum for reducing free radical damage induced by tobacco products and environmental pollutants comprising, as active ingredients, reduced glutathione, a source of selenium selected from the group consisting of elemental selenium, selenomethionine and selenocysteine, combined in a suitable carrier containing flavorings to produce a chewable gum in concentrations for reducing free radical damage induced by these tobacco products and other environmental pollutants to the oral cavity, pharynx and upper respiratory tract of a user and secondary smoker.

13. The gum of claim 12 wherein said gum further includes a gum base comprising approximately 40 to 60% by weight of the gum composition.

14. The gum of claim 13 wherein said gum base comprises an elastomer, a polyvinyl acetate polymer, an acetylated monoglyceride, a wax with melting point below approximately 60° C., an elastomer solvent, a plasticizer and a filler.

15. The gum of claim 12 comprising at least approximately 0.1 mg reduced glutathione, 20 mcgm of said source of selenium and further comprising at least approximately 10 mg ascorbic acid, 25 IU vitamin E and 1.0 mg of a

cysteine selected from the group consisting of L-cysteine and N-acetyl cysteine.

16. The gum of claim 12 further comprising a sweetener selected from the group consisting of xylitol, lactitol, sucrose, lactose and a saccharide.

17. A lozenge for reducing free radical damage induced by tobacco products and environmental pollutants comprising as active ingredients, reduced glutathione and a source of selenium selected from the group consisting of elemental selenium, selenomethionine and selenocysteine combined in a suitable carrier to enable the lozenge to slowly dissolve in a user's mouth releasing said active ingredients in concentrations for reducing free radical damage induced by tobacco products and other environmental pollutants to the oral cavity, pharynx and upper respiratory tract of a user and secondary smoker.

18. The lozenge of claim 17 wherein said lozenge is sized so that a user would be administered said active ingredients in from one to four lozenges to supply a recommended daily allowance of said active ingredients.

19. The lozenge of claim 18 wherein said lozenge is sized so that said one to four lozenges are sized to provide a user with approximately at least 40 mg reduced glutathione, 25 mcgm selenomethionine, 30 mg ascorbic acid, 15 1U alpha tocopherol, 2500 IU retinyl acetate and 10 mg L-cysteine, daily.

20. A chewable tablet for reducing free radical damage induced by tobacco products and environmental pollutants comprising as active ingredients, reduced glutathione and a source of selenium selected from the group consisting of elemental selenium, selenomethionine and selenocysteine combined with a suitable carrier to enable the chewable tablet to be masticated by teeth of the user releasing said active ingredients in concentrations for reducing free radical damage induced by tobacco products and other environmental pollutants to the oral cavity, pharynx and upper respiratory tract of a user and secondary smoker.

21. The chewable tablet of claim 20 wherein said chewable tablet is sized so that a user would be administered said active ingredients in from one to four chewable tablets to supply a recommended daily allowance of said active ingredients.

22. The chewable tablet of claim 21 wherein said chewable tablet is sized so that said one to four chewable tablets are sized to provide a user with approximately at least 40 mg reduced glutathione, 25 mcgm selenomethionine, 30 mg ascorbic acid, 15 IU alpha tocopherol, 2500 IU retinyl acetate and 10 mg L-cysteine, daily.

23. A method for reducing free radical damage induced by tobacco products and environmental pollutants comprising administering in a suitable carrier, in concentrations for effectively reducing said free radical damage to the oral cavity, pharynx and upper respiratory tract of a user a combination of reduced glutathione and a source of selenium as a member selected from the group consisting of elemental selenium, selenomethionine and selenocysteine in the form of a gel, gum, lozenge or chewable tablet.

24. The method of claim 23 wherein each of said gels, lozenges, tablets and gums contains at least approximately 0.5 mg. of said reduced glutathione.

25. The method of claim 23 wherein said gels, lozenges, tablets and gums contains at least approximately 5 mcgm. of said source of selenium.

26. The method of claim 23 wherein said gels, lozenges, tablets and gums further contain at least approximately 15 mg of vitamin C as ascorbic acid or as a derivative of ascorbic acid.

27. The method of claim 23 wherein said gels, lozenges, tablets and gums further contain at least approximately 10 IU of vitamin E as alpha tocopherol.

28. The method of claim 23 wherein said gels, lozenges, tablets and gums further contain superoxide dismutase.

29. The method of claim 23 wherein said gels, lozenges, tablets and gums further contain vitamin A.

30. The method of claim 23 wherein said gels, lozenges, tablets and gums further contain beta carotene.

31. The method of claim 23 wherein said gels, lozenges, tablets and gums further contain at least one amino acid selected from the group consisting of cysteine, methionine, taurine and arginine.

32. The method of claim 23 wherein said gels, lozenges, tablets and gums further contain a zinc salt.

33. The method of claim 32 wherein said zinc salt comprises zinc glutonate.

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(12) **United States Patent**
Georg et al.

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(45) **Date of Patent: Apr. 3, 2001**

(54) **SYNTHESIS OF EPOTHILONES**

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(52) **U.S. Cl.** **568/309**; 568/383
(58) **Field of Search** 560/129; 568/383, 568/309

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(57) **ABSTRACT**

Commercially feasible methods for synthesizing various epothilones precursors needed for the preparation of final epothilones are provided, including techniques for the synthesis of epothilone segment C using a stereoselective Noyori-type reduction, and the coupling of epothilone segments B and C using an aldol condensation reaction. The synthesis methods may be used to prepare naturally occurring segments as well as a large number of related analogs and homologs thereof. Final epothilones in accordance with the invention may also be of the naturally occurring variety (16-membered macrolides), while the homologs and analogs thereof are preferably up to 20-membered macrolides.

37 Claims, No Drawings

SYNTHESIS OF EPOTHILONES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is broadly concerned with methods for synthesizing various epothilone segments or precursors (either naturally occurring or analogs thereof) which can be used for the efficient synthesis of complete epothilones. More particularly, the invention pertains to such synthesis methods wherein, inter alia, the epothilone segment C is prepared using a unique Noyori reduction scheme, and epothilone segments B and C are connected via a novel aldol condensation reaction. These syntheses can be used to prepare the naturally occurring segments and a wide variety of corresponding analogs and homologs.

2. Description of the Prior Art

The epothilones (16-membered macrolides which were initially isolated from the myxobacterium *Sorangium cellulosum*) represent a class of promising anti-tumor agents, and have been found to be potent against various cancer lines, including breast cancer cell lines. These agents have the same biological mechanism of action as Taxol, an anti-cancer drug currently used as a primary therapy for the treatment of breast cancer. Other potential applications of the epothilones could be in the treatment of Alzheimer's disease, malaria and diseases caused by gram-negative organisms. Other cancers such as ovarian, stomach, colon, head and neck and leukemia could also potentially be treated. The epothilones also may have application in the treatment of arthritis.

In comparison to Taxol®, the epothilones have the advantage of being active against drug-resistant cell lines. Drug resistance is a major problem in chemotherapy and agents such as the epothilones have overcome this problem and hold great promise as effective agents in the fight against cancer.

In addition, the poor water solubility of Taxol® has led to the formulation of this drug as a 1:1 ethanol-Cremophor concentrate. It has been determined that the various hypersensitive reactions in patients such as difficulty in breathing, itchiness of the skin and low blood-pressure are caused by the oil Cremophor used in the formulation. The epothilones are more water soluble than Taxol® which has positive implications in its formulation. Further advantages of the epothilones include easy access to multi-gram quantities through fermentation procedures. Also the epothilones are synthetically less complex, thus structural modifications for structure activity relationship studies are easily accessible.

The epothilones exhibit their activity by disrupting uncontrolled cell division (mitosis), a characteristic of cancer, by binding to organelles called microtubules that are essential for this process. Microtubules play an important role in cell replication and disturbing the dynamics of this component in the cell stops cell reproduction and the growth of the tumor. Antitumor agents that act on the microtubule cytoskeleton fall into two general groups: (1) a group that inhibits microtubule formation and depolymerizes microtubules and, (2) a group that promotes microtubule formation and stabilizes microtubules against depolymerization. The epothilones belong to the second group and have displayed cytotoxicity and antimitotic activity against various tumor cell lines.

It has been demonstrated on the basis of in vitro studies that the epothilones, especially epothilone B, are much more effective than Taxol® against multi-drug resistant cell line

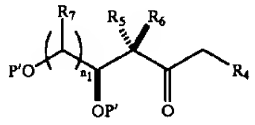
KBV-1. Preliminary in vivo comparisons with Taxol® in CB-17 SCID mice bearing drug-resistant human CCRF-CEM/VBL xenografts have shown that the reduction in tumor size was substantially greater with epothilone B in comparison to Taxol®.

In light of the great potential of the epothilones as chemotherapeutic agents, there is a need for techniques allowing the practical, large scale, economical synthesis thereof. Furthermore, there is a need for synthesis methods which facilitate the preparation of various homologs and analogs of the known epothilones, and those having affinity labels allowing study of the binding interactions of these molecules.

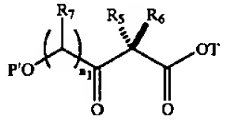
SUMMARY OF THE INVENTION

The present invention overcomes the problems outlined above, and provides various practical, commercially feasible synthesis routes for the production of important epothilone precursors or segments in high yield. The invention is particularly concerned with synthesis of the precursors or segments C, D (which is a combination of segments B and C) and vinyl halide epothilone precursors.

In a first aspect of the invention, an epothilone precursor of the formula



is synthesized using a Noyori reduction reaction. In the foregoing formula, n_1 is an integer from 0–4, R_4 is selected from the group consisting of H, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1–C10 alkoxy groups, R_5 and R_6 are each individually and respectively selected from the group consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1–C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups, R_7 is H or straight or branched chain C1–C10 alkyl groups, and P' is a protective group. The method comprises the steps of first providing a β-keto ester of the formula



where n_1, R_5, R_6, R_7 and P' are as defined above, and T is an alkyl group. This β-keto ester is then preferentially hydrogenated at the C3 keto group to form the corresponding hydroxyester. This is accomplished by reacting the β-keto ester with a hydrogenating agent in the presence of an asymmetric organometallic molecular catalyst comprising a metal atom or ion having one or more chiral ligands coupled thereto. The synthesis is completed by then converting the hydroxyester to the epothilone precursor.

More preferably, n_1 is an integer from 0–4, R_5, R_6 and R_7 are each individually and respectively selected from the group consisting of H and the straight and branched chain C1–C4 lower alkyls, and the protective group is benzyl. In terms of preferred process parameters, the hydrogenating agent is preferably H_2 and the hydrogenating step is carried

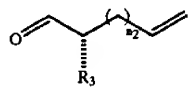
out at a pressure of from about 30–100 psi, more preferably 50–75 psi, and at a temperature of from about 40–100° C., more preferably from about 50–75° C. The reaction is normally allowed to proceed for a period of from about 12 hours to 5 days, and more usually for about 2–5 days. Typically, the reaction mixture is agitated during the hydrogenating step.

The catalyst used in the hydrogenation reaction is preferably one of the well-known Noyori catalysts such as $RuBr_2$(S)-binap. However, a variety of other catalysts of this type can also be employed. The catalyst is generally used at a level of from about 1–25 mol % in the reaction mixture.

In order to complete the reaction sequence, the hydroxyester resulting from the Noyori reduction is converted to the epothilone precursor segment C. A number of routes can be used to effect this conversion. Preferably, however, the conversion involves: (1) removing the P' protecting group from the hydroxyester to form a diol; (2) protecting the oxygen atoms of the diol, forming a protected diol; (3) reducing the ester function of the protected diol to a primary alcohol; (4) oxidizing the primary alcohol to the corresponding aldehyde; (5) reacting the aldehyde with a Grignard reagent having the R_4 group coupled thereto to form a secondary alcohol; and (6) oxidizing the secondary alcohol to form the final epothilone precursor.

Preferably, the P' removal step involves reacting the hydroxyester with hydrogen in the presence of a catalyst (e.g., $Pd(OH)_2$ or Pd/C) at a pressure of from about 40–100 psi. The oxygen atom protecting step comprises reacting the diol with TBS chloride in a compatible solvent (i.e., one that will not interfere with the desired reaction) at a temperature of from about 40–100° C. for a period of from about 30–60 hours. The ester function reduction step is preferably carried out by reacting the protected diol with the reducing agent DIBAL-H at a temperature of from about −20 to −85° C. The oxidation of the primary alcohol is carried out most conveniently using 4-methylmorpholine N-oxide and a catalytic amount of tetrapropylammonium perruthenate. The Grignard reaction serving to attach the R_4 group is entirely conventional and well within the skill of the art; likewise, the final oxidation of the secondary alcohol is trivial using the aforementioned oxidation procedure, i.e., NMO and TPAP.

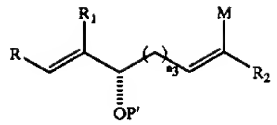
In another aspect of the invention, a method is provided for the production of epothilone precursor D, which is a combination of segments B and C. Segment C is of course produced as outlined above. Segment B is of the formula



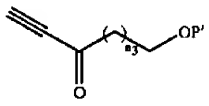
where n_2 is an integer from 1–4, and R_3 is selected from the group consisting of H, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1–C10 alkoxy groups. This segment can be efficiently produced using known techniques.

The segments B and C are connected by first reacting the segment C precursor with a base to form an enolate, followed by reacting the enolate with the segment B. These reactions are generally carried out by initially cooling the base to a temperature of about −75° C., adding the segment C precursor and elevating the temperature of the mixture to about −40° C., then recooling the mixture to at least about −75° C. and adding the precursor segment B thereto.

The invention also is concerned with a method of synthesizing vinyl halide epothilone precursors having the general formula



where n_3 is an integer from 1–4, R is selected from the group consisting of C4–C8 cycloalkyl, and substituted and unsubstituted aromatic and heteroaromatic groups, R_1 and R_2 are each individually and respectively selected from the group consisting of H, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1–C10 alkoxy groups, P' is a protecting group, and M is either bromine or iodine. This reaction involves first providing an alkynyl ketone of the formula



wherein n_3 and P' are as previously defined. Thereafter, the alkynyl ketone is asymmetrically reduced to create the alcohol form of the alkynyl ketone. This alcohol form is then reacted with a reagent system selected from the group consisting of$(R_1)_3Al$ and zirconocene dichloride or stannyl cupration reagent and R_1-halide to form a vinyl metal species. The vinyl metal species is then reacted with an aryl or vinyl halide to form an allyl alcohol. This allyl alcohol is then converted to the vinyl halide epothilone precursor.

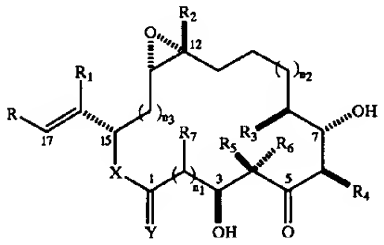
Normally, the asymmetric reduction step involves creating the reduced form of the alkynyl ketone and the resulting alcohol is protected using TBS as a protecting agent. The R_1-halide is selected from the group consisting of R_1Br and R_1I. The conversion step preferably includes the step of initially converting the allyl alcohol to an alkynyl stannane, reducing the stannane with chlorohydridozirconocene to form a 1,1-dimetallo Zr—Sn species. The dimetallo species is then hydrated to form a vinyl stannane, which is then quenched with either iodine or bromine. Alternately, the conversion step may be accomplished by transmetallating the dimetallo species with an organocuprate, quenching with an alkyl-R_2-OTf, and final quenching with either iodine or bromine incorporating the R_2 group.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

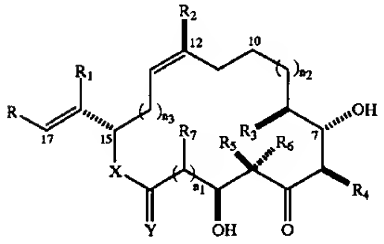
The molecular architecture of representative epothilones (Formulae A-B) reveals three essential domains. These include the two chiral domains, namely the C1–C8 polypropionate region and the C12–C15 region, and the achiral spacer C9–C11 which unites the chiral domains. Additional structural features include a thiazole moiety, the C16 double bond, a methyl group at C4 and a cis-epoxide moiety (C12–C13) in the epothilones of Formula A. In the following formulae A and B, n_1 is an integer from 0–4, n_2 and n_3 are each respectively integers from 1–4, R is selected from the group consisting of C4–C8 cycloalkyl, and substituted and unsubstituted aromatic and heteroaromatic groups, R_1, R_2, R_3 and R_4 are each individually and respectively selected from the group consisting of H, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1–C10 alkoxy groups, R_5 and R_6 are each individually and respectively selected from the group

consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1–C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups, R_7 is H, or straight or branched chain C1–C10 alkyl groups, X is either oxygen or NH, and Y is either oxygen or H_2.

FORMULA A



FORMULA B



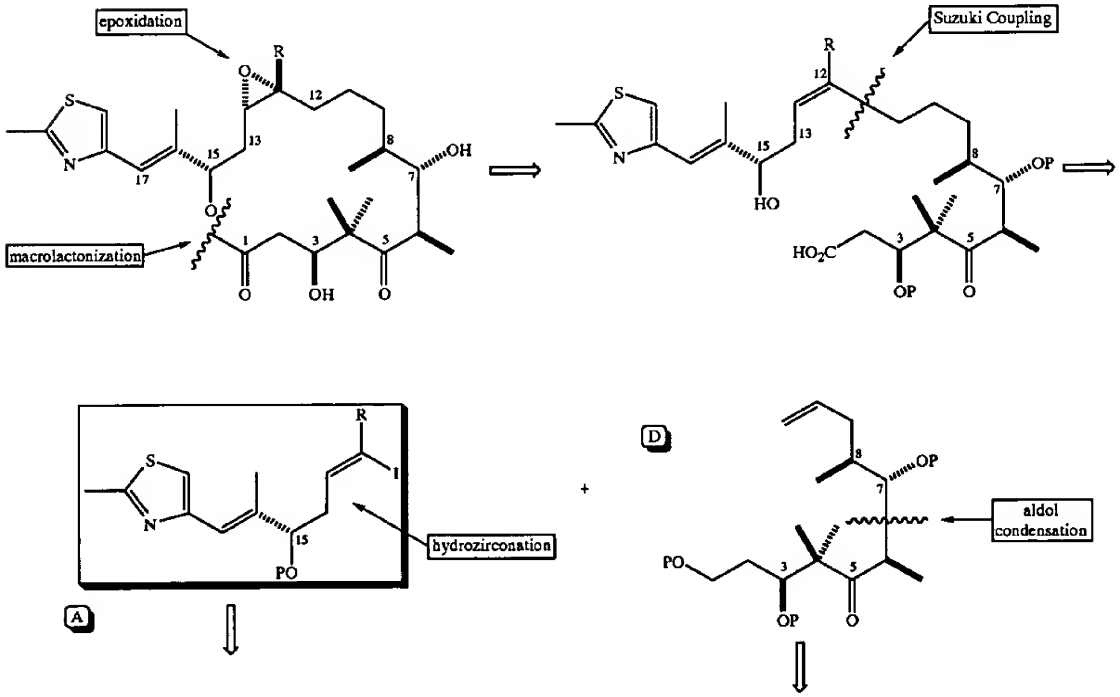
Scheme 1 below outlines a retrosynthetic analysis respecting the total synthesis of the epothilones of Formula A in accordance with the invention, where each n_1 and n_2 equal 1, R is 2-methyl-thiazol-4-yl, R_1 is methyl, R_2 is H or methyl, R_3, R_4, R_5 and R_6 are methyl, R_7 is H, and X and Y are oxygen. Standard epoxidation and macrolactonization strategies are used for the formation of the C12–C13 epoxide moiety and the 16-membered macrolide. The analysis for other analog epothilones of Formula A is identical, and also for the epothilones of Formula B and its analogs, with the epoxidation step being omitted. Included among the novel features of this synthesis are the following:

A novel route to the C1–C6 segment labeled C in Scheme 1 that utilizes a stereoselective hydrogenation reaction, i.e., a Noyori reduction.

A novel connection of segments C and the C7–C11 segment B utilizing a diastereoselective aldol reaction that renders the required diastereomer.

A novel route to the C12–C20 segment A which provides maximum control over the geometries of the two double bonds i.e., C12–C13 and the C16–C17 double bond. This is achieved by using stereo- and regioselective hydro/carbometalation reactions starting from alkyne precursors.

Scheme 1



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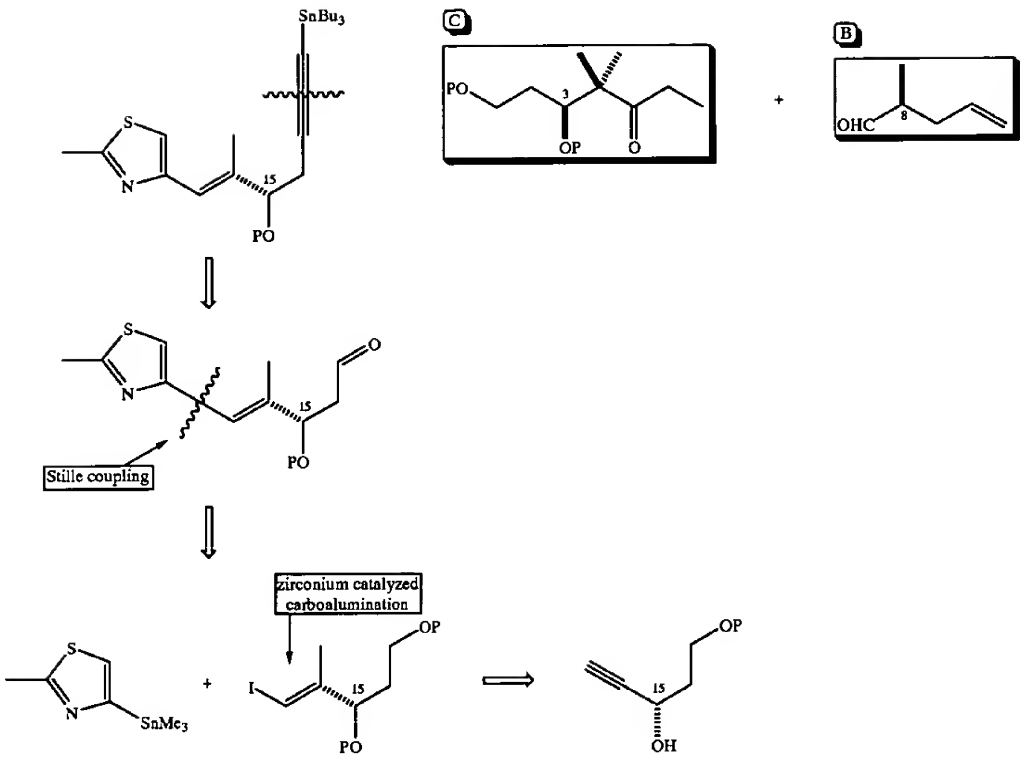


Synthesis of Segment C (C1–C6 of Formula A)

The synthesis of segment C has been accomplished via two unique and complementary routes, detailed in Schemes 2 and 3 below, which illustrates the synthesis of the naturally occurring segment C. A novel step in the synthesis of the C1–C6 segment utilizes the Noyori hydrogenation of β-keto ester 4 to generate the requisite stereochemistry at C3. This Noyori hydrogenation (Noyori, R. et al., in Asymmetric Hydrogenation of β-Keto Carboxylic Esters. A Practical, Purely Chemical Access to β-Hydroxy Esters in High Enantiomeric Excess, 109 J. Am. Chem. Soc. 5856–5858 (1987)) provides the required enantiomer with high selectivities (92–95% enantiomeric excess). The use of a Noyori hydrogenation reaction permits large, commercial scale production of segment C.

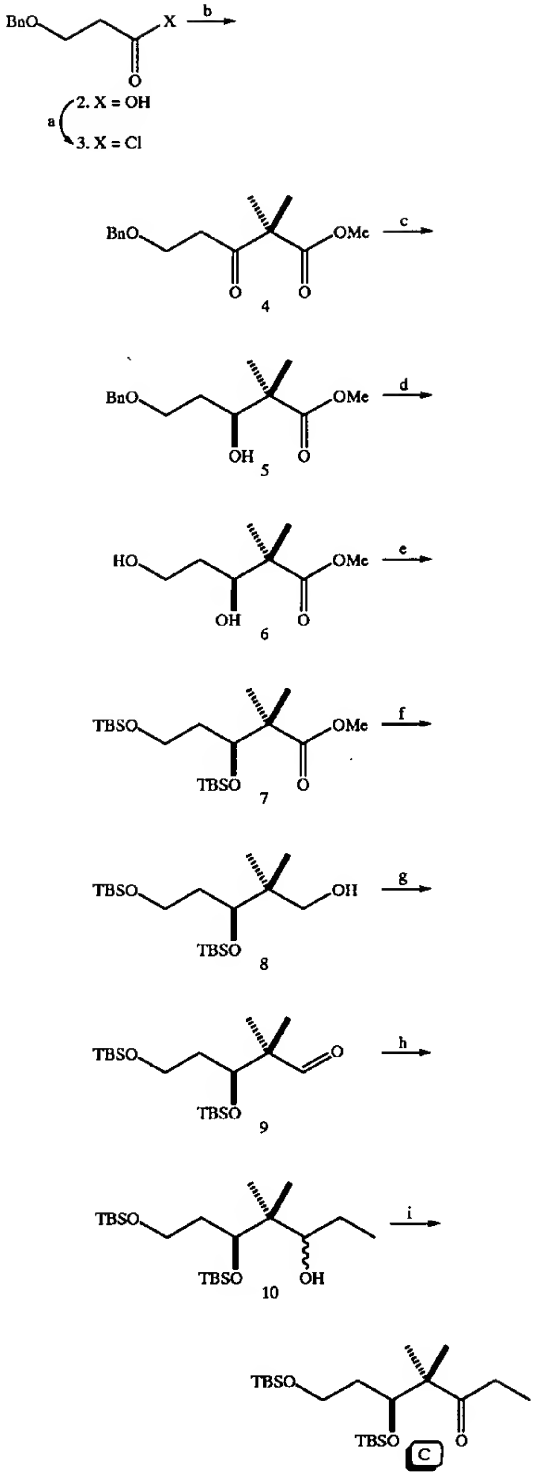
The required β-keto ester 4 is obtained in two steps from the readily available starting material 3-benzyloxypropionic acid (2). Asymmetric hydrogenation of 4 in methanol using $RuBr_2$(S)-binap as catalyst at 60 psi gives the β-hydroxyester 5 in 71–85% yield (92–95% ee). Deprotection of the benzyl ether and bis-silylation of the resultant diol 6 provides ester 7. The ester is reduced to the known primary alcohol 8 using DIBAL-H. The alcohol is then oxidized to the known aldehyde 9 using a previously unreported oxidation procedure. The aldehyde is then reacted with EtMgBr using a reported procedure (Claus, et al., Synthesis of the C1–C9 Segment of Epothilons, Tetrahedron Lett., 38:1359–1362 (1997)) to give the known secondary alcohol 10 in 65% yield. This alcohol is then oxidized to the C1–C6 segment C using TPAP and NMO.

In summary, although segment C is a key synthon in previously reported total syntheses (Nicolaou, et al., Total Syntheses of Epothilones A and B via a Macrolactonization-Based Strategy, J. Am. Chem. Soc., 119:7974–7991 (1997)) of the epothilones, the synthetic route utilizing the asymmetric Noyori hydrogenation is unique.

The alternate route toward segment C allows for the introduction of affinity labels and modifications at the C4 position as shown in Scheme 3. Applying the Noyori reduction to the known unsubstituted β-keto ester 11 provides a building block that can be used for the modifications at C4 of the epothilones. This Scheme accordingly allows for modification of the epothilones and gives a more general route to introduce a variety of substituents at this position.

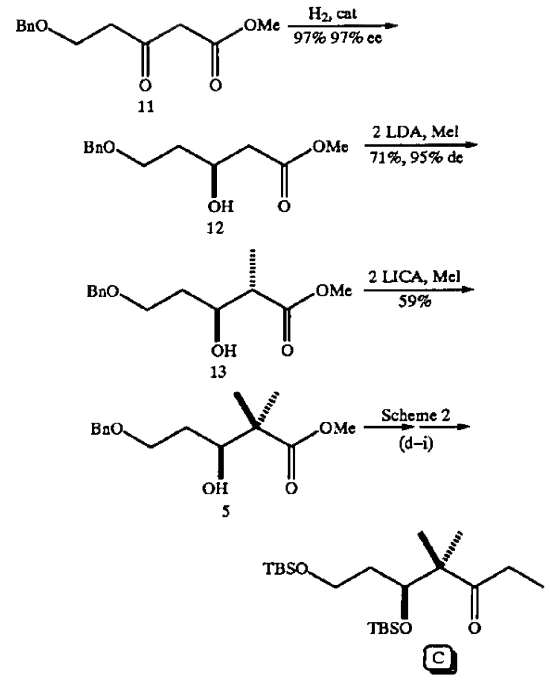
Thus, the Noyori hydrogenation of β-keto ester 11 yields the known β-hydroxy ester 12 (Ali, et al., Formal Syntheses of Cryptophycin 1 and Arenastatin A, Tetrahedron Lett., 38:1703–1706 (1997)) in 97% yield (in 97% enantiomeric excess). The Frater alkylation of β-hydroxy ester 12 yields the previously reported α-methyl analogue 13 (Ali, et al., Formal Syntheses of Cryptophycin 1 and Arenastatin A, Tetrahedron Lett., 38:1703–1706 (1997)) in 71% yield (98% diastereomeric excess). A second Frater alkylation of hydroxy ester 13 gave bis-dimethyl derivative 5 in 59% yield which was then converted to epothilone segment C by the chemistry shown in Scheme 2. At this stage, other substituents such as benzyl, allyl and other C1–C6 alkyl groups can be introduced by using other electrophiles in the second Frater alkylation in place of iodomethane. The novel aspect about this alternate route to segment C is the ability to alter the substituents at the C4 position of the epothilones using the aforementioned Frater alkylation strategy.

Scheme 2



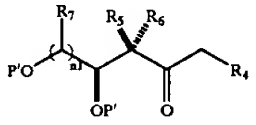
a) $(COCl)_2$, CH_2Cl_2, 99%; b) methyl isobutyrate, LICA, THF, -78° C., 65%; c) $RuBr_2$, (S)-binap, H_2, MeOH, 65 psi, 70° C., 71%, (92% ee); d) H_2, $Pd(OH)_2$, THF, 90%; e) TBSCl, imidazole, DMF, 60° C., 78%; f) DIBAL-H, CH_2Cl_2, -78° C., 95%; g) TPAP, NMO, 4 Å MS, CH_2Cl_2, 85%; h) EtMgBr, Et_2O, -78° C., 65%; i) same as g, 92%.

Scheme 3



The invention makes it possible to synthesize several analogs of segment C with various chain elongations and/or substitutions at C2 and substitutions at the α-carbon relative to the keto group. It also allows for, as mentioned before, modifications at the carbon atom between the keto and the protected secondary hydroxy group with other groups. These chain extensions and substitutions are illustrated by a general Formula C, wherein n_1 is as defined above, R_5, R_6 and R_7 are as defined previously, and P' is a protecting group, especially TBS or p-methoxybenzylidene acetal. The synthesis of these modified derivatives can be achieved utilizing chemistry exemplified in the synthesis of segment C in Schemes 2 and 3 respectively. These modified segments can then be utilized in the total synthesis of various analogs of epothilones.

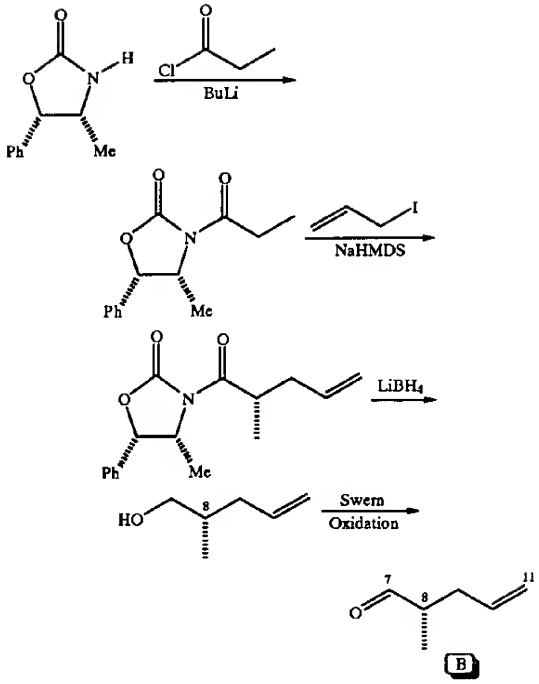
FORMULA C



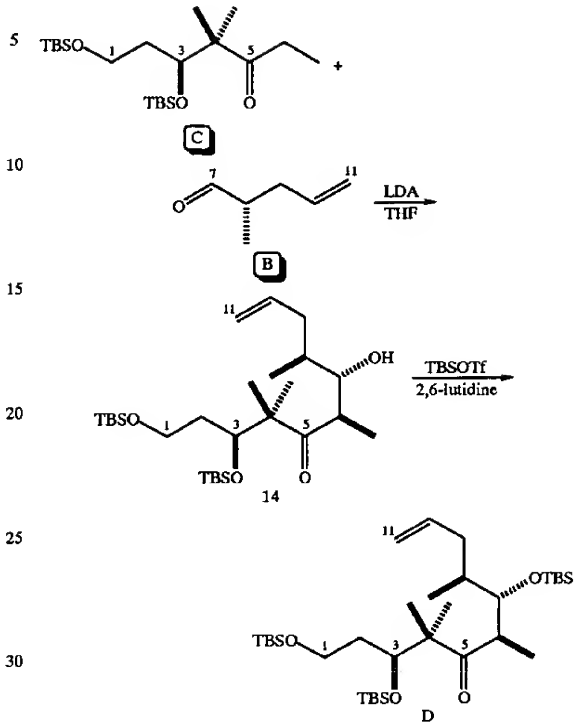
Synthesis of Segment B (C7–C11 of Formula A)

The synthesis of the C7–C11 segment B is preferably achieved using previously reported chemistry (Lin, Efficient Total Syntheses of Pumiliotoxins A and B, Applications of Iodide-Promoted Iminium Ion-Alkyne Cyclization in Alkaloid Construction, J. Am. Chem. Soc., 118:9062–9072 (1996)) and is outlined in exemplary Scheme 4, which is precursor of a naturally occurring epothilone.

Scheme 4

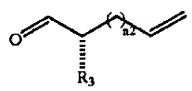


Scheme 5



This synthesis can also be used to introduce various chain-elongations on this segment and to introduce various other substituents at C-8. These modifications can be illustrated by Formula D, wherein n_2 and R_3 are as defined previously. Their synthesis can be achieved using chemistry exemplified in the synthesis of segment B in Scheme 4. Again, these modified segments can then be utilized in the total synthesis of various analogs of epothilones.

FORMULA D



Synthesis of Segment D (C1–C11 of Formula A) via Aldol Reaction

The connection of the two segments C and B utilizes a highly diastereoselective aldol reaction, exemplified in Scheme 5 showing the connection of the two precursors B and C of a naturally occurring epothilone. When the C1–C6 ketone segment C is treated with a base, for example lithium diisopropylamide and the resultant enolate reacted with C7–C11 aldehyde segment B, a single desired diastereomer 14 was observed in 21% yield (unoptimized). This diastereselectivity is believed to arise from a favorable nonbonding interaction between the C10–C11 double bond and the carbonyl group of the aldehyde that gives rise to the desired diastereomer. After the connection is made, the resultant secondary alcohol is protected as the corresponding tert-butyldimethylsilyl ether.

Similar chemistries would apply for the connection of modified segments C and B of the type discussed previously and emplified by Formulae C and D.

Proposed Synthesis of Segment A (C12–C20 of Formula A)

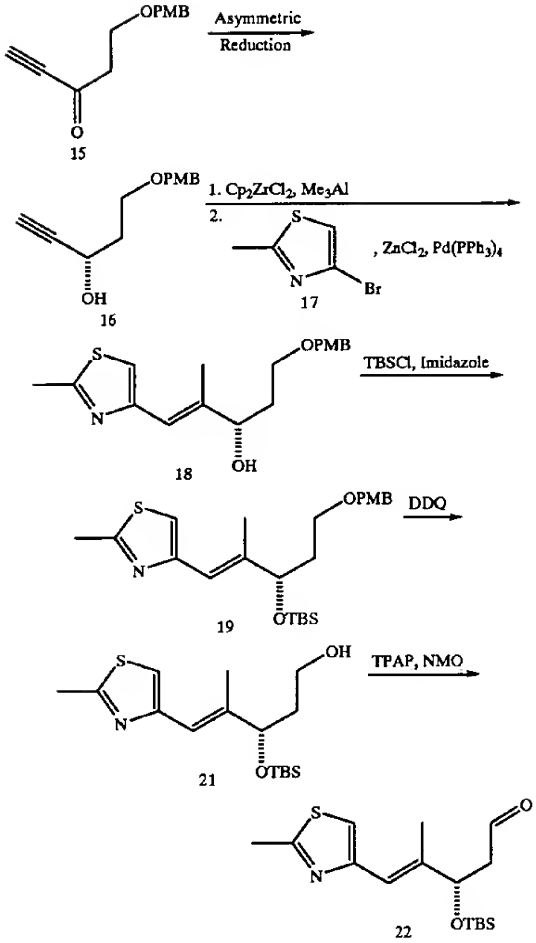
The invention also provides a new route to the C12–C20 segment (segment A of the naturally occurring epothilone), and corresponding analogs thereof. This involves new ways to set the C16–C17 trisubstituted double bond and the C12–C13 cis-double bond, which serves as precursor to the cis-epoxide at C12–C13 in the epothilones.

Stereoselective Construction of C16–C17 of Trisubstituted Olefin and Introduction of Thiazole in Formula A

The introduction of the thiazole moiety draws upon zirconium-catalyzed carboalumination chemistry (Wipf, Rapid Carboalumination of Alkynes in the Presence of Water, Agnew. Chem., Int. Ed. Engl., 32:1068–1071 (1993)) wherein a C16–C17 alkyne bond in an appropriately functionalized C13–C17 propargylic alcohol 16 (Scheme 6) is subjected to methylalumination in the presence of zirconocene dichloride ($C_{p2}ZrCl_2$). The resultant alkenylalane is coupled with 2-methyl-4-bromothiazole 17 in the presence of zinc chloride under Pd(0) catalysis to access the trisubstituted E-olefin 19 stereoselectively following the protection of the alcohol 18 as the OTBS-ether.

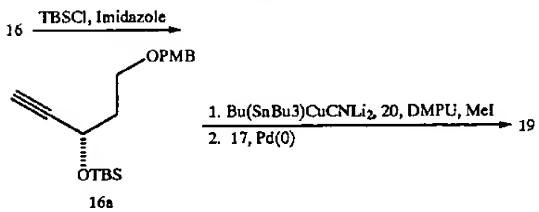
The chiral propargylic alcohol 16 is obtained via the asymmetric reduction of the readily available alkynyl ketone 15. This is exemplified in Scheme 6, which illustrates the synthesis of the precursor for the naturally occurring epothilone. After the introduction of the thiazole moiety, the known primary alcohol 21 is revealed by deprotection of the PMB ether 19 and then oxidized to the previously reported (Mulzer, J., et al. Easy Access to the Epothilone Family—Synthesis of Epothilone B, Tetrahedron Lett., 39:8633–8636 (1998)), C13–C20 aldehyde 22.

Scheme 6



Alternately, a stannylcupration-methylation methodology (Harris, et al., Synthetic Approaches to Rapamycin. 3. Synthesis of a C1–C21 Fragment, Synlett, pp. 903–905 (1996)) can be used in order to introduce the trisubstituted olefin. Thus the O-TBS ether 16a (Scheme 7) of propargylic alcohol 16 on treatment with the stannylcuprate reagent 20 followed by methylation with iodomethane provides the corresponding stannane which is then coupled under Stille conditions with the bromothiazole 17 to yield the olefin 19.

Scheme 7



The synthesis of 2-methyl-4-bromothiazole 17 from the known 2,4-dibromothiazole (Reynaud, et al., Sur une Nouvelle Synthese du Cycle Thiazolique, Bull. Soc. Chim. Fr., 295:1735–1738(1962)) is outlined in Scheme 8.

Scheme 8

The zirconium-catalyzed methylalumination strategy constitutes a novel route to construct the C16–C17 double bond and to introduce the thiazole ring. The novelty lies in the use of a chiral propargylic alcohol like 16 in the carbometalation reaction followed by the direct introduction of the thiazole unit.

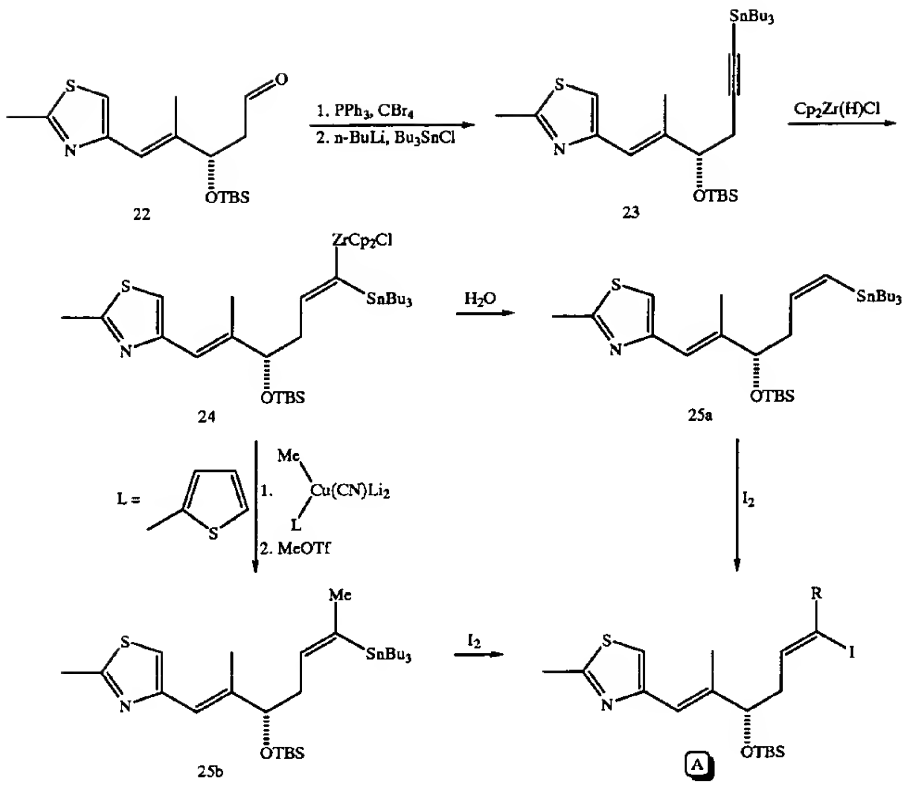
This methodology also allows for the introduction of various substituents and chain elongations on the C12–C20 segment A. Thus starting with analogs of the ketone 15 in Scheme 6, a variety of chain-elongated derivatives of segment A can be produced. Also carrying out an ethylalumination (Et_3Al) in place of methylalumination ($AlMe_3$) (Scheme 6) allows the introduction of an ethyl group (Et) at C16. In the same context, other groups can also be introduced using the alternate stannylcupration-alkylation method by replacing iodomethane with other electrophiles in this reaction shown in Scheme 7. In addition, the thiazole ring can be replaced by other cyclic, aromatic and heteroaromatic rings by using other vinyl or aromatic/heteroaromatic halides in place of 2-methyl-4-bromothiazole 17 in the coupling reaction following either the carboalumination or stannylcupration strategy exemplified in Schemes 6 and 7 respectively.

Stereoselective Construction of the C12–C13 cis-Olefinic Bond of Formula A

The goals in the construction of the C12–C13 Z-olefinic, were to design a method providing maximum control over the olefin geometry and to furnish common intermediates in the synthesis of both epothilones A and B. The introduction of affinity labels at C-12 was also a consideration.

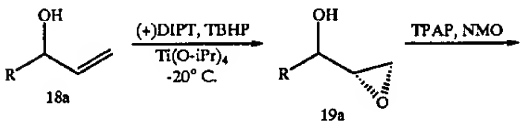
The C12–C13 olefin can be constructed in the form of Z-vinyl iodides I that can be obtained from vinylstannanes with defined configurations. The vinyl stannanes will be accessed by using known chemistry reported by Lipshutz et al., Preparation of Z-Vinylstannanes via Hydrozirconation of Stannylacetylenes, Tetrahedron Lett., 33:5861–5864 (1992); Lipshutz, et al., Hydrozirconation/Transmetalation of Acetylenic Stannanes. New 1,1-Dimetallo Reagents, Inorganica Chimica Acta, 220:41–44 (1994), which utilizes a 1,1-dimetallo species as a stereodefined 1,1-vinyl dianion synthon. An exemplary synthesis is given in Scheme 9, for the precursor to a naturally occurring epothilone, and starts with a Corey-Fuchs reaction (PPh_3, CBr_4) of the known aldehyde 22, followed by base-induced elimination and quenching of the lithium acetylide with tributyltin chloride (Bu_3SnCl) to yield alkynylstannane 23. The 1,1-dimetallo species 24 is generated by hydrozirconation of the alkynyl stannane 23 using chlorohydridozirconocene (Schwartz reagent). An aqueous quench would provide Z-vinylstannane 25a or alternatively, selective transmetalation with a higher order cuprate, followed by addition of an electrophile (MeOTf in case of epothilone B) to the resultant species provides the α-substituted vinylstannane 25b with high stereoselectivity. The Z-vinylstannanes 25a and 25b can then be transformed to the corresponding vinyl iodides I utilizing iodine with retention of configuration.

Scheme 9



An alternative route to the synthesis of alkynylstannane 23 (Scheme 9a) which would allow for incorporation of different substituents at the C16 carbon involves the asymmetric epoxidation of secondary alcohol 18a under the Sharpless conditions using (-)-diisopropyl tartrate, tert-butyl hydroperoxide and titanium isopropoxide to give epoxide 19a. The alcohol function on the epoxide can be oxidized with TPAP, NMO to give ketone 20a which can be reacted with Wittig reagents containing thiazole or other aromatic/heteroaromatic rings to give the corresponding trans-olefins. The terminal epoxide in this olefin can then be opened with trimethylsilyl acetylide to give secondary alcohol 22a. The trimethylsilyl group can then be substituted for a trialkyl stannyl group on treatment of 22a with TBAF and bis-tributyltin oxide and the obtained product treated with TBSCl to give compound 23.

Scheme 9a



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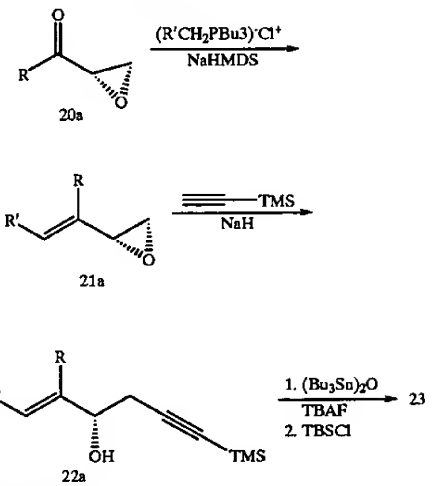
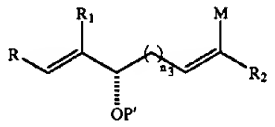


The foregoing chemistries can be used for the synthesis of analog precursors as well. Such analogs are best illustrated by Formula E, wherein n_3, R, R_1 and R_2 are as defined previously. Again all of these modified segments can then be utilized in the total synthesis of various analogs of epothilones.

In summary, although the vinyl iodides E are previously reported (20,21) compounds, the method to synthesize it from the known aldehyde 22 is different from conditions reported in other total syntheses of epothilones. In addition, the above mentioned hydrozirconation reactions provide precise control over the geometry of the C12–C13 olefin bond. Also the use of other electrophiles in the transmetalation reaction with the intermediate species 24 allows for the synthesis of various analogs.

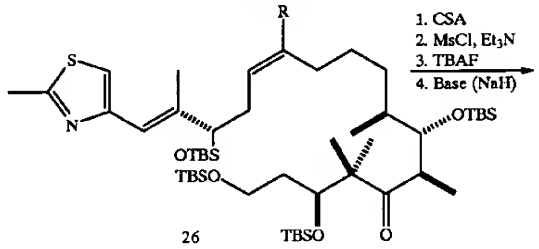
FORMULA E

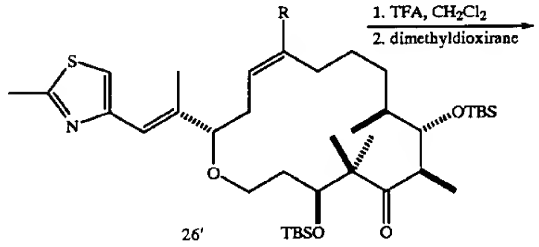


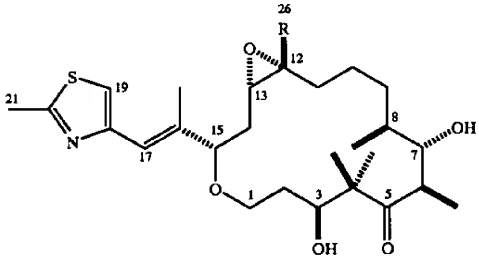
Two other epothilone derivatives of special interest may be synthesized in accordance with the invention. In one such derivative the lactone functional group is replaced with an ether functionality and in the other a lactam functionality is used in lieu of the lactone functional group. Thus in the first derivative, and referring to Formula A, X is O,Y is H_2, n_1, n_2, and n_3 are 1, R is 2-methyl-thiazol-4-yl, R_1 is methyl, R_2 is H or methyl, and R_3, R_4, R_5 and R_6 are methyl. In the second derivative, the only change is that X is NH and Y is O. These could be synthesized by the reaction sequences shown in Schemes 10 and 11. Thus selective deprotection at C1 by camphorsulfonic acid (CSA) (Scheme 10), formation of the mesylate derivative of the corresponding primary alcohol, selective deprotection of the C15 TBS ether and base-induced cyclic ether formation should provide compounds 26'. Again, the final stages in the synthesis would involve the deprotection of both the TBS groups from the macrolides (TFA, CH_2Cl_2) and the diastereoselective epoxidation of the C12–C13 double bond with epoxidizing agents such as dimethyldioxirane to give the ether derivatives 29 and 30.

For the lactam formation (Scheme 11) again compound 26 could be selectively deprotected at C-1 followed by sequential oxidation of the primary alcohol first under Swern conditions followed by $NaClO_2$—NaH_2PO_4 would furnish the known acids. These known acids can be converted to their allyl esters and then the TBS ether at C15 can be deprotected selectively. Mitsunobu inversion of these alcohols and azide formation via the corresponding mesylates will provide the azides with the correct stereochemistry at C15. Reduction of the azides (PPh_3, H_2O) followed by salt formation of the amine will provide 32. Deprotection of the allyl esters ($Pd(PPh_3)_4$, base) followed by macrolactamization (HBTU) will provide the lactams 33. Again, deprotection of both the TBS groups from the macrolides (TFA, CH_2Cl_2) and the diastereoselective epoxidation of the C12–C13 double bond with epoxidizing agents such as dimethyldioxirane would give the lactam derivatives 34 and 35.

Scheme 10

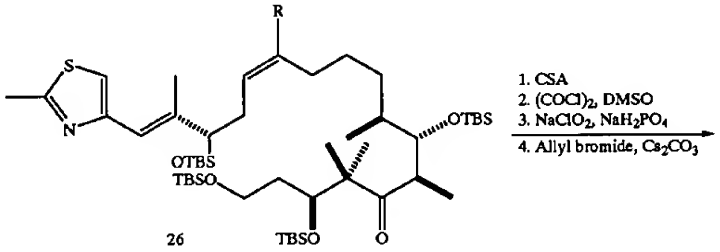






R = H, (29)
R = Me, (30)

Scheme 11



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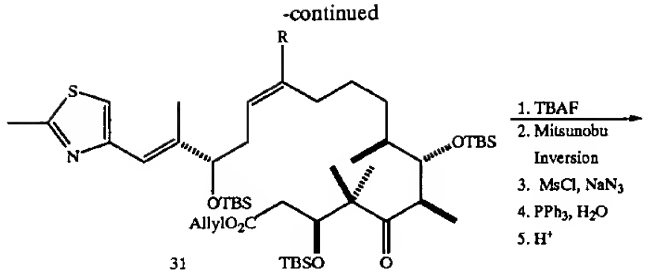
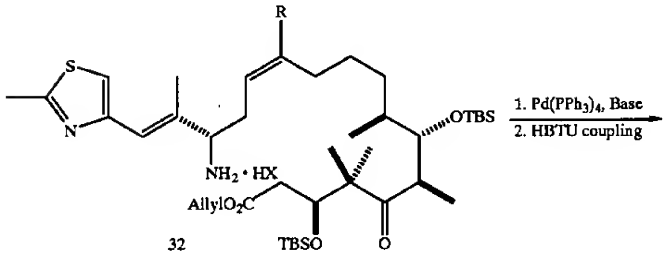
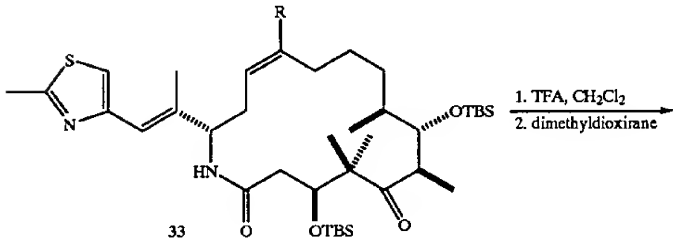


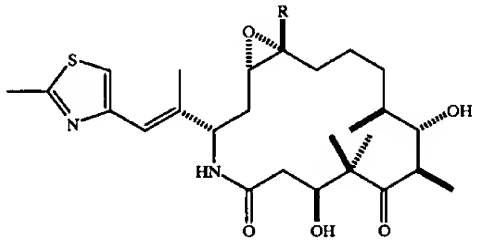
31



32



33



R = H (34)
R = Me (35)

Representative C4–C8 cycloalkyl, substituted and unsubstituted aromatic and heteroaromatic groups, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, C1–C10 alkoxy groups, and heterocyclic groups useful in the formation of epothilone analogs are set forth below.

C4–C8 cycloalkyl groups: cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl.

Substituted and unsubstituted aromatic groups: phenyl, phenyl groups substituted at any position with C1–C4 straight or branched chain alkyls, C1–C4 alkoxy groups, halogens, amines, amides, azides, sulfides, carboxylic acids and their derivatives, and hydroxides.

Substituted and unsubstituted heteroaromatic groups: thiazoles, pyrroles, furans, thiophenes, oxazoles and pyrridines, and imidazoles.

C1–C10 straight and branched chain alkyl groups: methyl, ethyl, propyl, butyl, isopropyl, isobutyl, isopentyl, octyl, nonyl, and t-butyl.

Substituted and unsubstituted benzyl groups: benzyl, benzyl groups substituted at any position with C1–C4 straight or branched chain alkyls, C1–C4 alkoxy groups, halogens, amines, amides, azides, sulfides, carboxylic acids and their derivatives, and hydroxides.

C1–C10 alkoxy groups: methoxy, ethoxy, propoxy, butoxy, isopropoxy, t-butoxy, and nonoxy.

Heterocyclic groups: piperidines, furans, pyrroles, oxazolines, and thiophenes.

The following examples set forth various syntheses of the type described previously. It is to be understood, however, that these examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

EXAMPLE 1

The synthesis of segment C was accomplished via two unique and complementary routes which are detailed in Schemes 2 and 3. One novel step in the synthesis of the C1–C6 segment utilizes Noyori hydrogenation as detailed in Noyori, R. et al., Asymmetric Hydrogenation of β-Keto Carboxylic Esters. A Practical, Purely Chemical Access to β-Hydroxy Esters in High Enantiomeric Excess, 109 J. Am. Chem. Soc., pp. 5856–5858 (1987). The Noyori hydrogenation of β-keto ester 4 in Scheme 2 generates the requisite stereochemistry at C3. This Noyori hydrogenation provides the required enantiomer with high selectivities (92% enantiomeric excess). It is a versatile reaction and has found numerous applications in the synthesis of biologically active natural products and is also amenable to large scale synthesis.

The following is a detail of the procedures that are outlined in Scheme 2. The required β-keto ester 4 is obtained in two steps from the readily available starting material 3-benzyloxypropionic acid (2) as described by Davis et al., Nonracemic α-fluoro aldehydes: Asymmetric synthesis of 4-deoxy-4-fluoro-d-arabinopyranose, J. Org. Chem., 62:7546–7547 (1997), the teachings of which are hereby incorporated by reference. Isopropylcyclohexylamine (7.3 mL, 44.6 mmol, 1.5 eq.) was dissolved in 40 mLTHF. The temperature was then lowered to −30° C. and n-butyllithium (16.1 mL, 38.6 mmol, 1.3 eq.) was added dropwise and stirred for 30 minutes. Next, the temperature was raised to 0° C. for 15 minutes and then cooled to −78° C. for 15 minutes. Methyl isobutyrate (3.75 mL 32.7 mmol, 1.1 eq.) was dissolved in 5 mL THF and added dropwise. The resulting mixture was stirred 30 minutes. 3-Benzyloxypropionyl chloride (5.0 g, 29.7 mmol, 1 eq) in 5 mL THF was then added dropwise. This reaction mixture was stirred for one hour until the starting material completely disappears by thin layer chromatography (TLC) (80:20 hexanes/EtOAc). The reaction mixture was then quenched with 20 mL 20% HCl and raised to room temperature. Next, the reaction mixture was extracted 3 times with ether and the combined organic phases were washed twice with sodium bicarbonate and once with brine. The combined aqueous layers were cross-extracted twice with ether. The organics were combined, dried with Na_2SO_4, and concentrated under reduced pressure. Purification was achieved via column chromatography utilizing a silica gel, hexane/EtOAc gradient which resulted in 5.1 g (65% yield) of β-keto ester 4.

Asymmetric hydrogenation of β-keto ester 4 in methanol using $RuBr_2$(S)-binap as catalyst at 65 psi gave the β-hydroxyester 5 in 85% yield. This was done by the following process. Acetone and MeOH were distilled and stored over molecular sieves. Each was degassed five times using the freeze-thaw method and placed under argon. Noyori's Ruthenium catalyst (91 mg, 0.284 mmol, 1 eq.), preferably bis-(2-methylallyl)cycloocta-1.5-diene ruthenium (II) and (S)-BINAP ((s)-(−)-1,1'-bi-2naphthol) (177 mg, 0.284 mmol, 1 eq.) were combined in a Schlenk flask with 24 mL acetone and 2.0 mL HBr solution (0.25 mL 48% HBr, 5.1 mL acetone). The resulting mixture was stirred for 4 hours to allow the catalyst to form. The acetone was then removed under reduced pressure. Next, beta ketoester 4 (5.36 g, 20.3 mmol, 71.5 eq.) in 23 mL MeOH was degassed four times and then transferred to a Parr hydrogenation flask using MeOH. The catalyst was then rinsed into the Parr flask using MeOH. The hydrogenation reaction was conducted over 110 hours at 65 psi and 60° C. The contents were concentrated under reduced pressure and then taken up in ether. The reaction mixture was filtered twice to remove the catalyst and then concentrated. Final purification was obtained through column chromatography wherein the column contained silica gel and utilized a hexane/EtOAc gradient and yielded 4.57 g (85% yield).

Deprotection of the benzyl ether and bis-silylation of diol 6 provided ester 7. To produce diol 6, β-hydroxyester 5 (1.824 g, 6.86 mmol, 1 eq.) was dissolved in 20 mL THF and transferred to a Parr hydrogenation vessel under argon. $Pd(OH)_2$ (450 mg, 0.25 eq.) was added and the flask purged for an additional 10 minutes with argon. The hydrogenation reaction was conducted at 50 psi for 24 hours. Finally, the reaction mixture was washed through a frit, an ultrafine strainer in a filtration technique preferably frit with 300 mL EtOAc and concentrated under reduced pressure to give 1.18 g (90% yield) of diol 6.

In detail, ester 7 was made by taking diol 6 (450 mg, 2.84 mmol, 1 eq) dissolved in 3.15 mL DMF and adding imidazole (1.16 g, 17.04 mmol, 6 eq) which was stirred until dissolved. TBSCl (tert-Butyldimethylsilyl chloride) (1.28 g, 8.52 mmol, 3 eq) was added and the temperature was raised to 60° C. This reaction mixture was then stirred for 44 hours. Disappearance of the starting material was monitored by thin layer chromatography. The reaction was then quenched with H_2O and NH_4Cl. After quenching, the reaction mixture was extracted twice with ether. The ether layer was washed with $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. Purification via column chromatography (SiO_2, 95:5 hexane/EtOAC) gave 0.89 g or a 78% yield.

Primary alcohol 8 is the result of reducing ester 7 (1.89 g, 4.68 mmol, 1 eq) by dissolving it in 26 mL of CH_2Cl_2, cooling it to −78° C. and adding DIBAL-H (9.4 mL, 14 mmol, 1.5M in hexanes) dropwise. After stirring at this temperature (−78° C.) for one hour, the reaction was quenched with 10 mL of MeOH. Next, the reaction mixture was warmed to 0° C. and 10 mL of a saturated aqueous solution of potassium sodium tartarate was added. After stirring this mixture for 16 hours, the aqueous layer was extracted four times with CH_2Cl_2. The combined organics were dried over anhydrous sodium sulfate and concentrated to yield 1.59 g (92% yield) of the primary alcohol. Although this is a known alcohol, the synthetic route from this ester is novel.

Primary alcohol 8 was oxidized to aldehyde 9 by taking primary alcohol 8 (1.45 g, 3.86 mmol, 1 eq) and dissolving it in 25 mL CH_2Cl_2. Molecular sieves (4 Å, powdered) were added to aid in the removal of water and this mixture was stirred for 15 minutes. 4-methylmorpholine N-oxide (NMO) (0.77 g, 6.56 mmol, 1.7 eq) was then added and after stirring

for 30 minutes, tetrapropylammonium perruthenate (TPAP) (0.081 g, 0.23 mmol, 0.06 eq) was added. The reaction mixture was stirred for 16 hours at room temperature and then concentrated. It was then passed through a pad of 4:1 silica gel:Celite mixture (35 g) to yield 1.2 g (83% yield) of the aldehyde.

The aldehyde 9 is then reacted with EtMgBr using the procedure of Claus, E. et al., Synthesis of the C1–C9 Segment of Epothilons, 38 Tetrahedron Lett., 1359–1362 (1997), the procedure of which is hereby incorporated by reference, to give the known secondary alcohol 10 in 65% yield.

This secondary alcohol 10 is then oxidized to the C1–C6 segment C using the same procedure that was used to oxidize primary alcohol 8 to aldehyde 9. The alcohol 10 (50 mg, 0.124 mmol, 1 eq) was dissolved in 1 mL CH_2Cl_2 Molecular sieves (4 Å, powdered) were added and this mixture was stirred for 15 minutes. N-oxide NMO (25 g, 0.211 mmol, 1.7 eq) was then added and after stirring for 30 minutes, TPAP (3 mg, 0.0074 mmol, 0.06 eq) was added. The reaction mixture was stirred for 15 hours at room temperature and then concentrated. It was then purified by passing it through a column (20 g) of 5:1 silica gel:Celite mixture (5% EtOAC in hexane) to yield 46 mg (92%) of the ketone (segment C). Again although segment C is known the oxidation process used is different from conditions reported.

In summary, although segment C is a key synthon in previously reported total syntheses of the epothilones, the synthetic route utilizing the asymmetric Noyori hydrogenation is unique.

EXAMPLE 2

Scheme 3 outlines an alternate synthesis of β-hydroxyester 5 using known compound 13. This alternate route toward segment C allows for the introduction of affinity labels and modifications at the C4 position as shown in Scheme 3. Applying the Noyori reduction to the known unsubstituted β-keto ester 11 provides a building block that can be used for the modifications at C4 of the epothilones. There has only been one report so far of C4 modification on the epothilones and this method provides a more general route of introducing a variety of substituents at this position. This will also enable a more thorough study of the structure activity relationships of numerous C4 substituted analogues.

Thus, the Noyori hydrogenation of β-keto ester 11 yields the known β-hydroxyester 12, which was reported by Ali, et. al, Formal Syntheses of Cryptophycin 1 and Arenastatin A., 38 Tetrahedron Lett., 1703–1706 (1997), hereby incorporated by reference, in 97% yield (in 97% enantiomeric excess). The Frater alkylation of β-hydroxy ester 12 yields the previously reported α-methyl analogue 13 also previously reported by Ali et al. Formal Syntheses of Cryptophycin 1 and Arenastatin A., 38 Tetrahedron Lett., 1703–1706 (1997), hereby incorporated by reference, in 71% yield (98% diastereomeric excess). A second Frater alkylation of hydroxy ester 13 gave bis-dimethyl derivative 5 in 59% yield which was then converted to epothilone segment C by the chemistry shown in Scheme 2.

In detail, isopropylcyclohexylamine (0.71 mL, 4.32 mmol, 2.16 eq.) and 3.75 mL THF were stirred together at −25° C. n-BuLi (1.64 mL, 3.6 mmol, 1.8 eq.) was added dropwise over 15 minutes. The reaction mixture was stirred at room temperature for 15 minutes and then lowered to −78° C. Compound 13 (504 mg, 2 mmol, 1 eq.) in 2.5 mL THF was added to the reaction mixture. The temperature was gradually raised to −10° C. over 4 hours and then returned to −78° C. MeI (0.17 mL, 2.66 mmol, 1.33 eq.) in HMPA (0.26 mL) was added dropwise. The reaction was stirred at −78 ° C. for one hour and then stirred at room temperature for 16 hours. The reaction was quenched with 4 mL 10% HCl . The mixture was then extracted four times with CH_2Cl_2, dried over Na_2SO_4, and concentrated. Purification by column chromatography 160 g SiO_2 (hexane/EtOAc gradient) gave 312 mg (a 59% yield) of β-hydroxyester 5.

At this stage, other substituents such as benzyl, allyl and other alkyl groups can be introduced by using other electrophiles in the second Frater alkylation in place of iodomethane. The novel aspect about this alternate route to segment C is the ability to alter the substituents at the C4 position of the epothilones using the aforementioned Frater alkylation strategy. After synthesis of β-hydroxyester 5, segment C can be produced following the procedure outlined above in Scheme 2.

It should be noted that this invention makes it possible to synthesize several analogs of this C1–C6 segment with various chain elongations at C2 and substitution at C6 positions on the epothilones. It also allows for, as mentioned before, modifications at the C4 position with other groups such as aryl, heterocyclic, alkyl and branched alkyl. These chain extensions and substitutions are illustrated by Formula C. The synthesis of these modified derivatives can be achieved utilizing chemistry exemplified in the synthesis of segment C in Schemes 2 and 3 respectively. These modified segments can then be utilized in the total synthesis of various analogs of epothilones.

EXAMPLE 3

This example illustrates the synthesis of segment B. The synthesis of the C7–C11 segment B has been achieved using previously reported chemistry of Lin, et al., Efficient Total Syntheses of Pumiliotoxins A and B. Applications of Iodide-Promoted Iminium Ion-Alkyne Cyclization in Alkaloid Construction 118 J. Am. Chem. Soc., 9062–9072 (1996), the teachings of which are hereby incorporated by reference, and is outlined in Scheme 4. This synthesis can also be used to introduce various chain-elongations on this segment and to introduce various other substituents at C-8. These modifications can be illustrated by Formula D and their synthesis can be achieved using chemistry exemplified in the synthesis of segment B in Scheme 4. Again, these modified segments can then be utilized in the total synthesis of various analogs of epothilones.

EXAMPLE 4

This example, illustrated in Scheme 5, describes the synthesis of aldol adduct 14 followed by the aldol reaction of segment C, from examples 1 or 2, with segment B, from example 3. Aldol adduct 14 was then used to synthesize segment D.

The connection of the two segments C and B utilizes a highly diastereoselective aldol reaction (Scheme 5). When the C1–C6 ketone segment C was treated with a base, namely lithium diisopropylamide and the resultant enolate reacted with C7–C11 aldehyde segment B a single diastereomer 14 was observed in 21% yield (unoptimized). The remarkable diastereselectivity is speculated to arise from a favorable nonbonding interaction between the C10–C11 double bond and the carbonyl group of the aldehyde that gives rise to the desired diastereomer. This connection between these two particular segments using an aldol reaction is unprecedented. After the connection has been made,

the resultant secondary alcohol will be protected as the corresponding tert-butyldimethylsilyl ether D.

In detail, diisopropylamine (60 μL, 0.45 mmol, 1 eq) dissolved in 1 mL THF was cooled to −78 ° C. and n-BuLi (0.33 mL, 0.43 mmol, 0.95 eq) was added dropwise. After stirring at −78° C. for 15 minutes and at 0° C. for 30 minutes the reaction mixture was recooled to −78° C. The ketone (segment C) (0.1 84 g, 0.45 mmol, 1 eq) dissolved in 1 mL of THF was then added dropwise. This mixture was stirred at −78 ° C. for 15 minutes and then warmed to −40° C. over one hour. After recooling to −78° C, the aldehyde segment B (0.022 g, 0.23 mmol, 0.5 eq) dissolved in 0.5 mL Et_2O was added dropwise over 15 minutes. After 35 minutes at −78 ° C., the reaction was quenched with 2 mL saturated aqueous ammonium chloride and warmed to room temperature. The aqueous layer was extracted five times with Et_2O and the combined organics were dried over anhydrous magnesium sulfate. After concentration, preparative thin layer chromatography of the residue yielded 0.047 g (21% yield) of the desired diastereomer.

Forming segment D utilizes TBS protection of adduct 14. Aldol adduct 14 (30 mg, 0.06 mmol, 1 eq.) was diluted with 1.5 mL CH_2Cl_2 and the temperature was lowered to −78° C. 2,6-lutidine (50 μL, 0.42 mmol, 7 eq.) was added dropwise followed by tert-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (70 μL, 0.30 mmol, 5 eq.). The reaction was stirred for 15 minutes and then raised to 0° C. The reaction was complete after 3 hours and was quenched with 5 mL NH_4Cl. The mixture was extracted three times with CH_2Cl_2 and the combined organic layers were washed with brine, dried over $MgSO_4$ and then concentrated. Column chromatography (5% EtOAc in hexane) gave 34 mg (96% yield) of compound D.

EXAMPLE 5

This example illustrates the synthesis of segment A (the C-12–C-20 segment) and is outlined in Scheme 6. This involves new ways to set the C16–C17 trisubstituted double bond and the C12–C13 cis-double bond which serves as precursor to the cis-epoxide at C12–C13 in the epothilones. The methodology used to introduce the thiazole moiety draws upon zirconium-catalyzed carboalumination chemistry as described by Wipf, P. and Lim, S., Rapid Carboalumination of Alkynes in the Presence of Water, 32 Agnew. Chem., Int. Ed. Engl., 1068–1071 (1993), the teachings of which are hereby incorporated by reference. Using this chemistry, a C16–C17 alkyne bond in an appropriately functionalized C13–C17 propargylic alcohol 16 is subjected to methylalumination in the presence of zirconocene dichloride (Cp_2ZrCl_2). The chiral propargylic alcohol 16 is obtained via the asymmetric reduction of the readily available alkynyl ketone 15. A number of methods have been developed during the past years for the enantioselective reduction of α,β-alkynyl ketones. The resultant alkenylalane is coupled with 2-methyl-4-bromothiazole 17 in the presence of zinc chloride under Pd(0) catalysis as described by Negishi, E.-I., et al., in Double Metal Catalysis in the Cross-Coupling Reaction and Its Application to Stereo- and Regioselective Synthesis of Trisubstituted Olefins, 100 J. Am. Chem. Soc., 2254–2256 (1978) and Negishi, E.-I., in Palladium- or Nickel- Catalyzed Cross Coupling. A New Selective Method for Carbon-Carbon Bond Formation, 15 Acc. Chem. Res., 340–348 (1982), the teachings of which are hereby incorporated by reference. Scheme 8 illustrates the synthesis of 2-methyl-4-bromothiazole 17 which was synthesized by adding 9.0 mL ether and n-BuLi (1.8 mL, 2.96 mmol, 1.2 eq) and stirring at −78° C. for 30 minutes. The bromothiazole (0.60 g, 2.47 mmol, 1 eq.) in 3.5 mL of ether was added dropwise to the n-BuLi. After stirring for one hour, MeOTf (0.56 mL, 4.94 mmol, 2 eq.) was added dropwise and the reaction mixture was stirred for an additional 1.5 hours. The reaction was then quenched with $NaHCO_3$ and extracted 5 times with ether. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. 262 mg (60% yield) of the 2-methyl-4-bromothiazole 17 was obtained and carried on without further purification.

This coupling of the alkenylalane to 2-methyl-4-bromothiazole 17 permits access to the trisubstituted E-olefin 19 stereoselectively following the protection of the alcohol 18 as the OTBS-ether.

After the introduction of the thiazole moiety, the known primary alcohol 21(Mulzer et al., Easy Access to the Epothilone Family—Synthesis of Epothilone B, Tetrahedron Lett., 39:8633–8636 (1998) is revealed by deprotection of the PMB ether 19 and then oxidized to C13–C20 aldehyde 22, also previously reported by Mulzer, et al., Easy Access to the Epothilone Family—Synthesis of Epothilone B, 39 Tetrahedron Lett, 8633–8636 (1998), the teachings of which are hereby incorporated by reference.

The zirconium-catalyzed methylalumination strategy constitutes a novel route of constructing the C16–C17 double bond and introduction of the thiazole ring. The novelty lies in the unprecedented use of a chiral propargylic alcohol like 16 in the carbometalation reaction followed by the direct introduction of the thiazole unit.

This methodology also allows for the introduction of various substituents and chain elongations on the C12–C20 segment A. Thus starting with analogs of the ketone 15 in Scheme 6, a variety of chain-elongated derivatives of segment A can be accessed. Also carrying out an ethylalumination (Et_3Al) in place of methylalumination ($AlMe_3$) will allow the introduction of an ethyl group (Et) at C16. In the same context, other groups such as alkyl and benzyl groups can also be introduced using the alternate stannylcuprationalkylation method by replacing iodomethane with other electrophiles in this reaction shown below in Example 6 and illustrated in Scheme 7. In addition the thiazole ring can be replaced by other cyclic, aromatic and heteroaromatic rings by using other vinyl or aromatic/heteroaromatic halides in place of 2-methyl-4-bromothiazole 17 in the coupling reaction following either the carboalumination or stannylcupration strategy exemplified in Schemes 6 and 7 respectively.

These aforementioned modifications are best illustrated by Formula E. Again all of these modified segments can then be utilized in the total synthesis of various analogs of epothilones.

EXAMPLE 6

Scheme 7 illustrates an alternative way to introduce the trisubstituted olefin. This method utilizes the stannylcupration-methylation methodology described by Harris, L., et al., in Synthetic Approaches to Rapamycin 3. Synthesis of a C1–C21 Fragment, Synlett, 903–905 (1996), the methodology of which is hereby incorporated by reference. Thus the O-TBS ether 16a from Scheme 7 of propargylic alcohol 16 on treatment with the stannylcuprate reagent 20 followed by methylation with iodomethane would provide the corresponding stannane which would then be coupled under Stille conditions with the bromothiazole 17 to yield the olefin 19.

EXAMPLE 7

The synthesis of 2-methyl-4-bromothiazole 17 from the known 2,4-dibromothiazole is outlined in Scheme 8. This 2,4-dibromothiazole has been previously reported by Reynaud, P., Robba, M. and Moreau, R. C. in Sur une Nouvelle Synthese du Cycle Thiazolique, 295 Bull. Soc.

Chim. Fr., 1735–1738 (1962), the teachings of which are hereby incorporated by reference.

EXAMPLE 8

This example illustrates the stereoselective construction of the C12–C13 cis-olefinic bond, the process of which is outlined in Scheme 9. The method shown provides maximum control over the olefin geometry as well as furnishes common intermediates in the synthesis of both epothilones A and B. This method is also amenable to introduction of affinity labels at C-12.

The C12–C13 olefin is constructed in the form of Z-vinyl iodides A that can be obtained from vinylstannanes with defined configurations. The vinyl stannanes will be accessed by using the chemistry reported by Lipshutz et al., in Preparation of Z-Vinylstannanes via Hydrozirconation of Stannylacetylenes, 33 Tetrahedron Lett., 5861–5864 (1992) and Lipshutz, B. H. and Keil, R. in Hydrozirconation/Transmetalation of Acetylenic Stannanes. New 1,1-Dimetallo Reagents, 220 Inorganica Chimica Acta, 41– 44 (1994), the teachings of which are hereby incorporated by reference. The chemistry utilizes a 1,1 -dimetallo species as a stereodefined 1,1-vinyl dianion synthon.

The synthesis starts with a Corey-Fuchs reaction (PPh_3, CBr_4), as described by Corey, E. J. and Fuchs, P. L., A Synthetic Method for Formyl to Ethynyl Conversion, Tetrahedron Lett., 36:3769–3772 (1972) of the known aldehyde 22, followed by base-induced elimination and quenching of the lithium acetylide with tributyltin chloride (Bu_3SnCl) to yield alkynylstannane 23. The 1,1-dimetallo species 24 is generated by hydrozirconation of the alkynyl stannane 23 using chlorohydridozirconocene (Schwartz reagent). An aqueous quench provides Z-vinylstannane 25a or alternatively, selective transmetalation with a higher order cuprate, followed by addition of an electrophile (MeOTf in case of epothilone B) to the resultant species provides the α-substituted vinylstannane 25b with high stereoselectivity. The Z-vinylstannanes 25a and 25b can then be transformed to the corresponding vinyl iodides A utilizing iodine with retention of configuration.

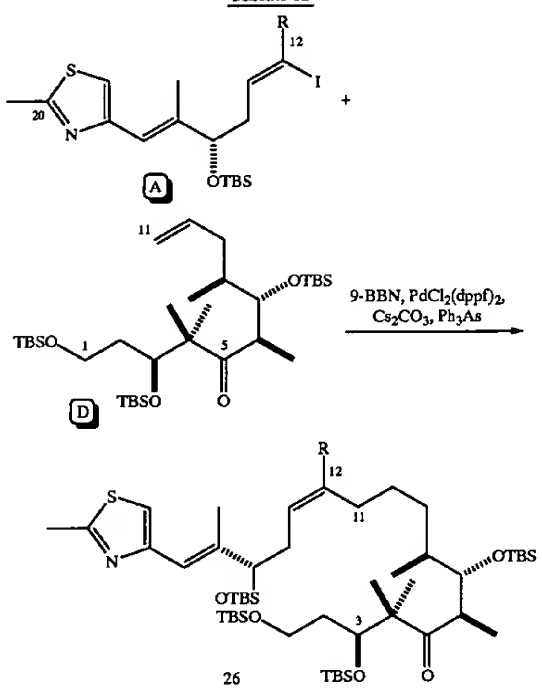
In summary, although the vinyl iodides A are previously reported compounds as evidenced by the teachings of Schinzer, D. et al., in Total Synthesis of (-)-Epothilone A, 36 Angew. Chem., Int. Ed. Engl., 523–524 (1997) and Schinzer, D., Bauer, A., and Scheiber, J., Synthesis of Epothilones: Stereoselective Routes to Epothilone B, Synlett, 861–864 (1998), the teachings of both are hereby incorporated by reference, the method to synthesize it from the known aldehyde 22 is different from conditions reported in other total syntheses of epothilones. In addition, the above mentioned hydrozirconation reactions provides precise control over the geometry of the C12–C13 olefin bond and this methodology constitutes a unique way to construct this double bond. Also the use of other electrophiles in the transmetalation reaction with the intermediate species 24 allows for the synthesis of various analogs modified at C12 position of the epothilones as illustrated in Formula E.

EXAMPLE 9

This example and the next (Example 10) illustrate C11–C12 bond construction including coupling of the C1–C11 (segment D) and C12–C20 (segment A) subunits and completion of the total synthesis.

Having defined all the requisite stereocenters and geometries, the stage is set for the union of the C1–C11 and the C12–C20 subunits. The C11–C12 bond connection is achieved by the B-alkyl Suzuki reaction of the C1–C11 olefin D with the vinyl iodides A shown in Scheme 10 to afford the precursors 26 for the synthesis of epothilone A and B. This B-alkyl Suzuki reaction is described by Miyaura, N., and Suzuki, A. in Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds, 95 Chem. Rev. 2457–2483 (1995), the teachings of which are hereby incorporated by reference. Thus, hydroboration of the olefin D with 9-BBN followed by coupling of the corresponding organoborane with the vinyl iodides A in presence of [$PdCl_2$ $(dppf)_2$] would yield the olefin 26.

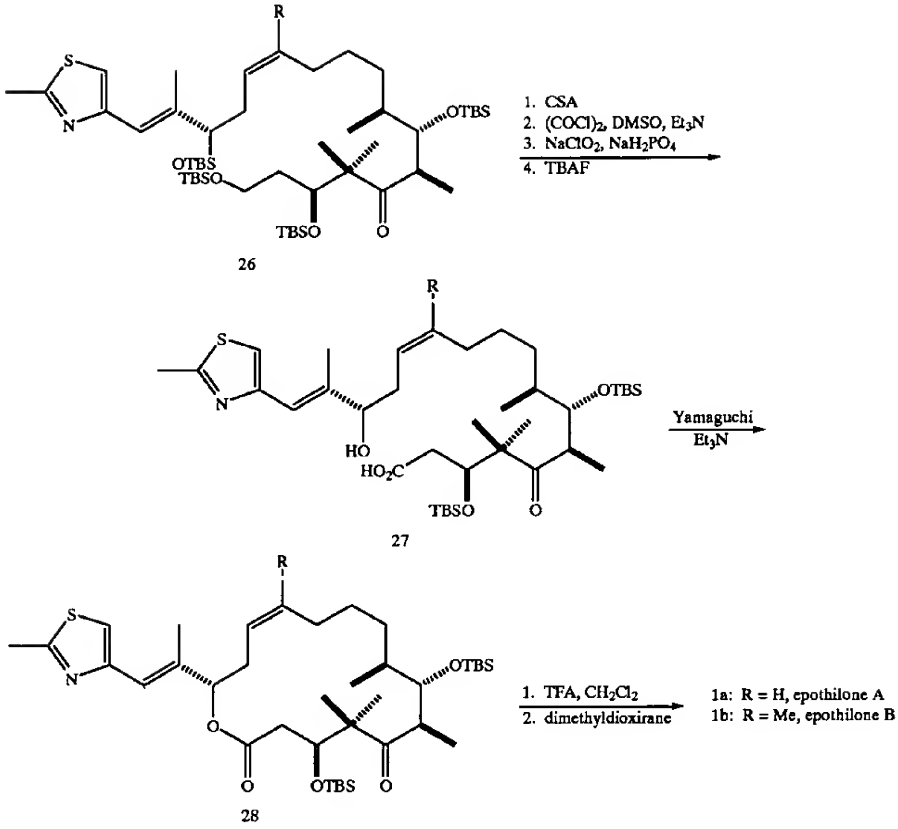
Scheme 12



EXAMPLE 10

Finally the conversion of the coupled C1–C20 products 26 to epothilones A and B is accomplished using the previously reported procedures of Nicolaou, K. C. et al.; in Total Syntheses of Epothilones A and B via a Macrolactonization-Based Strategy 119 J. Am. Chem. Soc., 7974–7991 (1997), the teachings of which are hereby incorporated by reference. Thus selective deprotection at C1 by camphorsulfonic acid (CSA), shown in Scheme 11, followed by sequential oxidation of the primary alcohol first under Swern conditions followed by $NaClO_2$—NaH_2PO_4 furnishes the known acids. Selective deprotection of the TBS ether at C15 using tetrabutylammonium fluoride yields the hydroxy acids 27. The key macrolactonization step is then carried out using the Yamaguchi method as described by Inanaga, J. et al., in A Rapid Esterification by Means of Mixed Anhydride and its Application to Large-ring Macrolactonization, 52 Bull. Chem. Soc. Jpn, 1989–1993 (1979), the teachings of which are hereby incorporated by reference, affording the known 16-membered macrolides 28. The final stages in the synthesis involve the deprotection of both the TBS groups from the macrolides 28 (TFA, CH_2Cl_2) and the diastereoselective epoxidation of the C12–C13 double bond with epoxidizing agents such as dimethyldioxirane or methyl(trifluoromethyl)dioxirane.

Scheme 13



As shown in Formulae C, D and E, various modified segments can be employed during the syntheses of the individual segments (C, B and A). All of these modified segments can then be connected using the bond connections (Aldol reaction and the B-alkyl Suzuki reaction) highlighted in this total synthesis. This would provide numerous homologs, analogs and affinity labels of the epothilones. All references noted herein are expressly incorporated by reference.

Acronym and Symbol Definitions

In order to facilitate the preceding discussion various acronyms and symbols have been used. These have the following definitions.

Ac	acetyl
Ar	aromatic
9-BBN	9-borabicyclo[3.3.1]nonane
(S)-BINAP	(S)-(-)-1,1'bi-2-naphthal
Bn	benzyl
BnO	benzyloxy
Bu	butyl
n-BuLi	n-butyl lithium
Cp_2ZrCl_2	zirconocene dichloride
$Cp_2Zr(H)Cl$	chlorohydridozirconocene
CSA	camphorsulfonic acid
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H	diisobutylaluminum hydride
DIEA	diisopropylethylamine

-continued

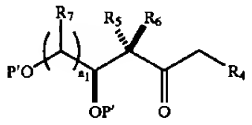
(+)DIPT	diisopropyl tartrate
DMF	N,N-dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)pyrimidinone
DMSO	dimethylsulfoxide
Et	ethyl
HBTU	O-(benzotriazol-1-y-1)-N,N,N',N' tetramethyluronium hexafluoro phosphate
HF	hydrogen fluoride
HMPA	hexamethylphosphoramide
LDA	lithium diisopropylamide
LHMDS	lithium hexamethyldisilazide
LICA	lithium isopropylcyclohexylamide
m-CPBA	meta-chloroperoxybenzoic acid
MDR	multi-drug resistant
Me	methyl
MeOTf	methyl triflate
MS	molecular sieves
MsCl	mesyl chloride
NaHMDS	sodium hexamethyldisilazide
NMO	4-methylmorpholine N-oxide
OTf	trifluoromethane sulfonate
$PdCl_2(dppf)_2$	dichloro[1,1'-Bis(diphenylphosphino)ferrocene] palladium II
$Pd(PPh_3)_4$	tetrakis (triphenylphosphine) palladium (0)
Ph	phenyl
PPh_3	triphenylphosphine
PMB	para-methoxybenzyl
Ru cat	bis-(2 methyl allyl) cycloocta-1,5-diene ruthenium (II)
TBAF	tetrabutylammonium fluoride
TBHP	tertbutyl hydroperoxide
TBS	tertiary-butyldimethylsilyl

-continued

TBSCl	tertiary-butyldimethylsilylchloride
TBSOTf	tertiary-butyldimethylsilyl triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
$Ti(O\text{-}iPr)_4$	titanium isopropoxide
TPAP	tetrapropylammonium perruthenate

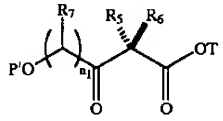
We claim:

1. A method of synthesizing an epothilone precursor of the formula



where n_1 is an integer from 0–4, R_4 is selected from the group consisting of H, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1–C10 alkoxy groups, R_5 and R_6 are each individually and respectively selected from the group consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1–C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups, R_7 is H or straight or branched chain C1–C10 alkyl groups, and P' is a protective group, comprising the steps of:

providing a β-keto ester of the formula



where n_1, R_4, R_5, R_6, R_7 and P' are as defined above, and T is an alkyl group;

preferentially hydrogenating the C3 keto group of said β-keto ester to form the corresponding hydroxyester by reacting the β-keto ester with a hydrogenating agent in the presence of an asymmetric organometallic molecular catalyst comprising a metal atom or ion having one or more chiral ligands coupled thereto; and

converting the hydroxyester to said epothilone precursor.

2. The method of claim 1, said hydrogenating agent being hydrogen.

3. The method of claim 1, said hydrogenating step being carried out at a pressure of from about 30–100 psi.

4. The method of claim 3, said pressure being from about 50–75 psi.

5. The method of claim 1, said hydrogenating step being carried out at a temperature of from about 40–100° C.

6. The method of claim 5, said temperature being from about 50–75° C.

7. The method of claim 1, said hydrogenating step being carried out for a period of from about 12 hours to 5 days.

8. The method of claim 7, said period being from about 2–5 days.

9. The method of claim 1, including the step of agitating the reaction mixture during said hydrogenation step.

10. The method of claim 1, said catalyst being present during said hydrogenation step at a level of from about 5 mol % to about 25 mol %.

11. The method of claim 1, said converting step comprising the steps of:

removing the P' protective group from said hydroxy ester to form a diol;

protecting the oxygen atoms of said diol to form a protected diol;

reducing the ester function of said protected diol to a primary alcohol;

oxidizing the primary alcohol to an aldehyde;

reacting the aldehyde with a Grignard reagent having said R_4 group coupled thereto to form a secondary alcohol; and

oxidizing the secondary alcohol to form said epothilone precursor.

12. The method of claim 11, said P' removal step comprising the steps of reacting said hydroxyester with hydrogen in the presence of a catalyst at a pressure of from about 40–100 psi.

13. The method of claim 12, said catalyst selected from the group consisting of $Pd(OH)_2$ and Pd/C.

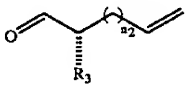
14. The method of claim 11, said oxygen atom protecting step comprising the steps of reacting said diol with TBS chloride in a solvent at a temperature of from about 40–100° C. for a period of from about 30–60 hours.

15. The method of claim 11, said ester function reducing step comprising the steps of reacting said protected diol with a reducing agent DIBAL-H at a temperature of from about −20 to −85° C.

16. The method of claim 11, said primary alcohol oxidizing step comprising the steps of reacting the primary alcohol with 4-methylmorpholine N-oxide and a catalytic amount of tetrapropylammonium perruthenate.

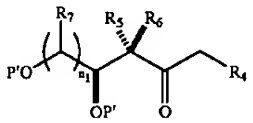
17. A method of connecting epothilone precursors comprising the steps of:

providing a first epothilone precursor of the formula



where n_2 is an integer from 1–4, and R_3 is selected from the group consisting of H, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1–C10 alkoxy groups;

providing a second epothilone moiety of the formula



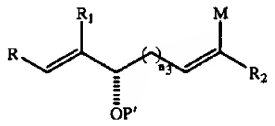
where n_1 is an integer from 0–4, R_4 is selected from the group consisting of H, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1–C10 alkoxy groups, R_5 and R_6 are each individually and respectively selected from the group consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1–C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups, R_7 is H, or straight or branched chain C1–C10 alkyl groups, and P' is a protective group, comprising the steps of:

reacting said second epothilone precursor with a base to form an enolate; and

reacting said enolate with said first epothilone precusor.

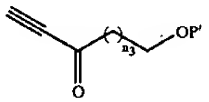
18. The method of claim **17**, said enolate-forming and enolate-first epothilone reactions comprising the steps of initially cooling said base to a temperature of at least about −75° C., and adding said second precursor to the cooled base, slowly elevating the temperature of the base-second precursor mixture to about −40° C., and thereafter recooling the mixture to at least about −75° C. and adding said first precursor thereto.

19. A method of synthesizing a vinyl halide epothilone precursor having the formula



where n_3 is an integer from 1–4, R is selected from the group consisting of C4–C8 cycloalkyl, and substituted and unsubstituted aromatic and heteroaromatic groups, R_1 and R_2, are each individually and respectively selected from the group consisting of H, C1–C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1–C10 alkoxy groups, P' is a protective group, and M is either bromine or iodine, comprising the steps of:

providing an alkynyl ketone of the formula



wherein n_3, M and R_2 are as previously defined;

asymmetrically reducing said alkynyl ketone to create the alcohol form of the alkynyl ketone;

reacting said alcohol form with a reagent system selected from the group consisting of $(R_1)_3Al$ and zirconocene dichloride or stannyl cupration reagent and R_1-halide to form a vinyl metal species;

reacting said vinyl metal species with an aryl or vinyl halide to form an allyl alcohol; and

converting said allyl alcohol to said halide epothilone precursor.

20. The method of claim **19**, said asymmetric reduction step comprising the step of creating the protected alcohol form of the alkynyl ketone.

21. The method of claim **19**, said R_1-halide being selected from the group consisting of R_1Br and R_1I.

22. The method of claim **19**, said conversion step including the step of initially converting the allyl alcohol to an alkynyl stannane, reducing said alkynyl stannane with chlorohydridozirconocene to form a 1,1-dimetallo Zr-Sn species.

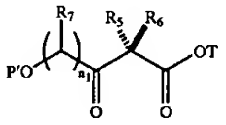
23. The method of claim **22**, including the steps of hydrating said dimetallo species to form a vinyl stannane, and then quenching the vinyl stannane with either iodine or bromine.

24. The method of claim **22**, including the steps of transmetallating said dimetallo species with an organocuprate and then quenching with an alkyl-R_2-OTf, and thereafter quenching with either iodine or bromine.

25. The method of claim **1**, said protecting group being selected from the group consisting of tert-butyldimethylsilyl protecting groups, benzyl protecting groups, and paramethoxybenzyl protecting groups.

26. A method of hydrogenating a β-keto ester comprising the steps of:

providing a β-keto ester of the formula



where n_1 is an integer from 0–4, R_5 and R_6 are each individually and respectively selected from the group consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1–C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups, R_7 is H or straight or branched chain C1–C10 alkyl groups, T is an alkyl group, and P' is a protective group; and

preferentially hydrogenating the C3 keto group of said β-keto ester to form the corresponding hydroxyester by reacting the β-keto ester with a hydrogenating agent in the presence of an asymmetric organometallic molecular catalyst comprising a metal atom or ion having one or more chiral ligands coupled thereto.

27. The method of claim **26**, said hydrogenating agent being hydrogen.

28. The method of claim **26**, said hydrogenating step being carried out at a pressure of from about 30–100 psi.

29. The method of claim **28**, said pressure being from about 50–75 psi.

30. The method of claim **26**, said hydrogenating step being carried out at a temperature of from about 40–100° C.

31. The method of claim **30**, said temperature being from about 50–75° C.

32. The method of claim **26**, said hydrogenating step being carried out for a period of from about 12 hours to 5 days.

33. The method of claim **32**, said period being from about 2–5 days.

34. The method of claim **26**, including the step of agitating the reaction mixture during said hydrogenation step.

35. The method of claim **26**, said catalyst being present during said hydrogenation step at a level of from about 5 mol % to about 25 mol %.

36. The method of claim **26**, said catalyst selected from the group consisting of $Pd(OH)_2$ and Pd/C.

37. The method of claim **26**, said protecting group being selected from the group consisting of tert-butyldimethylsilyl protecting groups, benzyl protecting groups, and paramethoxybenzyl protecting groups.

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